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# Study on Differentiate Procedure of Alkaline Phosphatase Test by Conventional Colorimetric Method and Optimized Standard Method in Pasteurized Milk

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**Abstract:** ***In order to determine pasteurized milk, one of the enzymes milk phosphates, is measured. A negative phosphates' result indicates that any pathogenic bacteria have been destroyed during pasteurization. If it is positive, it means the pasteurization process was inadequate and the milk may not be safe for human consumption and will have a short shelf life. In conventional method the milk samples are diluted with buffer at pH 10.2 and incubated at the temperature at 37ºC for 2hrs.Any alkaline phosphates present in the milk samples will liberate Para nitro phenol from artificially added disodium p-nitrophenyle phosphate which can be compared with the standard color disc. There are differences for measuring the phosphatase activity in between conventional method and Optimized Standard Method. So Chowdhury AP[1] et.al., applied " Optimized Standard Method" according to the recommendation of the German clinical chemistry association (Deutsche Gesellschaft fur Klinische Chemie, DGKC) at the purpose of showing its better performance than conventional procedure with in short time.***

**Keywords:** Alkaline Phosphatase, Chromogenic substances, DGKC method, Mastitis Test.

**Objectives:** Study on differentiate Results of ALP analysis from Milk samples (Raw, Pasteurized and UHT) distinguishing between conventional method and newly applied DGKC method.

## 1. Introduction

Pasteurization is a method of Heat treating milk to improve its storage qualities and destroy pathogenic bacteria. Milk is heated to 65°C for 30 minutes or 72°C for 15 minutes followed by rapid cooling to bellow 10°C; the method was devised by the French Microbiologist Louis Pasteur (1822-1895).

A simple phosphates' test is recommended to determine whether milk has been properly pasteurized or not. Milk has the alkaline Phosphatase inactivated by the time /temperature combinations applied during pasteurization. To determine about the complete pasteurization of milk if it is free from micro organisms contaminating raw milk, a chromogenic substances is added. The alkaline phosphates present in the milk will hydrolyze the substrate producing a color which can be compared to standards to determine whether the milk is acceptable or not. The pasteurized milk should have less than one coli form per ml and after 5 days storage at 6°C; its count at 21°C should be less than $10^5$ cfu/ml.

When milk is pasteurized at 63ºC for 30 min in batch pasteurizer or 72ºC for 15 seconds in heat exchanger, continuous flow pasteurizers, all pathogenic bacteria destroyed , there by rendering milk safe for human consumption. Simultaneously various enzymes present in milk, and which might affect its flavor, are destroyed.

## 2. Materials and Methods

### 2.1 Samples Collection

In the present study two types of milk samples were collected - **Raw milk** samples were collected from 40 (forty) healthy cows of Senowara Dairy farm, Chittagong, and **Pasteurized milk** samples - Farm Fresh, Milk Vita, Aarong and Pran were collected from four UHT milk suppliers -

### 2.2 Samples Preparation

Raw milk of healthy cows from Senowara Dairy Farm was denoted as Sample RM1; half of the raw milk samples were pasteurized in a home pasteurizer and denoted them as RM2. Commercially pasteurized (UHT) milks - Farm Fresh, Milk Vita, Aarong and Pran were denoted as PM1, PM2, PM3 and PM4 respectively.

### 2.3 Screening of Raw milk Samples for Mastitis and Somatic Cells

Raw Milk samples collected from **Senowara Dairy Farm** were used to determine whether they were having Mastitis and somatic cells. For this purpose, milk samples were studied details. The California Mastitis Test (CMT) is a rapid, accurate, cow side test that helps to determine somatic cell counts (SCC) of raw milk from a specific cow. Los Angeles County Board of Supervisors has developed the CMT.

**2.3.1. California Mastitis Test (CMT)**

The test was developed to determine the presence of subclinical mastitis in raw milk sample from individual quarters. The mixture of Sodium Dodecyle Benzosulfonate - 30 gm and Bromocresol purple - 1 gm was used as CMT reagent. Each teat of the cow was washed with alcohol prior to squire the milk. A small amount of milk sample (approximately ½ teaspoon) from each quarter was collected into a plastic paddle that has 4 shallow cups marked as A, B, C and D. An equal amount of CMT reagent was added to the milk in each cup of the paddle. The paddle was rotated to mix the contents for approximately 10 seconds and score while continuing to rotate the paddle as the reaction disappears within 20 seconds. The test result was read quickly.

**2.3.2. Somatic cell count (SCC)**

Somatic Cell Count (SCC) is used as an indicator of the quality of raw milk (i.e., its suitability to make high-quality milk products). Somatic cells are primarily white blood cells (i.e., leukocytes). The number of somatic cells may increase as a result of udder infection (e.g., mastitis) or teat/udder injury and varies due to many factors, including the cow's age, lactation stage, season and stress.

**2.3.3 Standard Results of CMT Scores** (Los Angeles County Board of Supervisors)

**Table1:**

| CMT Score | Avg. Somatic Count (Cells / ml) | Description of reaction |
|---|---|---|
| Negative | ≤ 100,000 | No thickening, homogeneous. |
| Subclinical | ≤ 300,000 | Slight thickening. Reaction disappears in 10 seconds. |
| Clinical | ≤ 900,000 | Distinct thickening, no gel formation. |
| Sub acute | ≤ 2,700,000 | Thickens immediately, begins to gel, levels in the bottom of cup. |
| Acute | ≤ 8,100,000 | Gel is formed, surface elevates, with a central peak above mass. |

**Table 2**

| *Samples* | *CMT Score* | *Avg. Somatic Count (Cells / ml)* | *Standard* | *Description of reaction* |
|---|---|---|---|---|
| RM1 | Acute | 8,500,000 | 8,100,000 | Gel is formed, surface elevates, with a central peak above mass. |
| RM2 | Subclinical | 270,985 | 300,000 | Slight thickening. Reaction disappears in 10 seconds |
| RM3 | Subclinical | 278,654 | 300,000 | Slight thickening. Reaction disappears in 10 seconds |
| RM4 | Subclinical | 289,765 | 300,000 | Slight thickening. Reaction disappears in 10 seconds |
| RM5 | Negative | 80,794 | 100,000 | No thickening, homogeneous |
| RM6 | Clinical | 897,654 | 900,000 | Distinct thickening, no gel formation. |
| RM7 | Subclinical | 277,239 | 300,000 | Slight thickening. Reaction disappears in 10 seconds |
| RM8 | Negative | 100,000 | 100,000 | No thickening, homogeneous |
| RM9 | Acute | 8,233,456 | 8,100,000 | Gel is formed, surface elevates, with a central peak above mass. |
| RM10 | Negative | 67,894 | 100,000 | No thickening, homogeneous |
| RM11 | Negative | 87,596 | 100,000 | No thickening, homogeneous |
| RM12 | Subclinical | 199,876 | 300,000 | Slight thickening. Reaction disappears in 10 seconds |
| RM13 | Negative | 76,892 | 100,000 | No thickening, homogeneous |
| RM14 | Negative | 84,676 | 100,000 | No thickening, homogeneous |
| RM15 | Negative | 99,305 | 100,000 | No thickening, homogeneous |
| RM16 | Clinical | 879,343 | 900,000 | Distinct thickening, no gel formation. |
| RM17 | Sub acute | 2,657,865 | 2,700,000 | Thickens immediately, begins to gel, levels in the bottom of cup. |
| RM18 | Sub acute | 2,776,543 | 2,700,000 | Thickens immediately, begins to gel, levels in the bottom of cup. |
| RM19 | Negative | 99,847 | 100,000 | No thickening, homogeneous |
| RM20 | Subclinical | 298,788 | 300,000 | Slight thickening. Reaction disappears in 10 seconds |
| RM 21 | Sub acute | 2,567,898 | 2,700,000 | Thickens immediately, begins to gel, levels in the bottom of cup. |
| RM22 | Sub acute | 2,665,933 | 2,700,000 | Thickens immediately, begins to gel, levels in the bottom of cup. |
| RM23 | Subclinical | 234,660 | 300,000 | Slight thickening. Reaction disappears in 10 seconds |
| RM24 | Negative | 76,543 | 100,000 | No thickening, homogeneous |
| RM25 | Sub acute | 2,577,681 | 2,700,000 | Thickens immediately, begins to gel, levels in the bottom of cup |
| RM26 | Acute | 8,245,678 | 8,100,000 | Gel is formed, surface elevates, with a central peak above mass. |
| RM27 | Sub acute | 2,656,443 | 2,700,000 | Thickens immediately, begins to gel, levels in the bottom of cup. |
| RM28 | Negative | 87,652 | 100,000 | No thickening, homogeneous |
| RM29 | Sub acute | 2,567,768 | 2,700,000 | Thickens immediately, begins to gel, levels in the bottom of cup. |
| RM30 | Negative | 80,765 | 100,000 | No thickening, homogeneous |
| RM31 | Acute | 8,123,987 | 8,100,000 | Gel is formed, surface elevates, with a central peak above mass. |
| RM32 | Subclinical | 255,632 | 300,000 | Slight thickening. Reaction disappears in 10 seconds |
| RM33 | Negative | 97,654 | 100,000 | No thickening, homogeneous |
| RM34 | Subclinical | 277,984 | 300,000 | Slight thickening. Reaction disappears in 10 seconds |
| RM35 | Sub acute | 2,699,832 | 2,700,000 | Thickens immediately, begins to gel, levels in the bottom of cup. |
| RM36 | Negative | 89,987 | 100,000 | No thickening, homogeneous |
| RM37 | Clinical | 899,349 | 900,000 | Distinct thickening, no gel formation. |
| RM38 | Sub acute | 2,633,458 | 2,700,000 | Thickens immediately, begins to gel, levels in the bottom of cup. |
| RM39 | Negative | 65,432 | 100,000 | No thickening, homogeneous |
| RM40 | Acute | 8,346,342 | 8,100,000 | Gel is formed, surface elevates, with a central peak above mass. |

In this method verified results were studied then mastitis negative samples denoted for rejection. Following the tested mastitis samples should to take for Pasteurization by dint of Home Pasteurizer machine. Recommended samples should to take for Pasteurization process due to presence of Mastitis causing Microorganisms of milk.

**Table 3:**

| Biochemical Types | Microorganisms | Substrate acted upon & end products |
|---|---|---|
| Acid Producers | *Streptococcus lactis.*<br>*Streptococcus cremoris.*<br>*Lactobacillus spp.* | Lactose fermented to lactic acid and other products such as carbon dioxide, ethyl alcohol, acetic acid, etc.<br>Lactose is fermented to lactic acid and other products. |
| | *Microbacterium lactium*<br>*Escherichia coli*<br>*Enterobacter aerogenes*<br>*Micrococcus luteus* | Lactose is fermented to lactic acid. Heat resistant (80-85˚)C<br>Lactose fermented to mixture of end products such as acid, gases, & neutral products.<br>Weakly fermentative of lactose; weakly proteolytic. Moderately heat resistant (60-63˚ )C. |
| Gas Producers | *Coliforms spp., Clostridium spp., Butyricum spp.,Torula cremoris.* | Lactose fermented with accumulation of gas (mixture of carbon- di-oxide & hydrogen). |
| Ropy or Stringy Fermentaters | *Alcaligenes viscolactis, Enterobacter aerogenes Streptococcus cremoris* | Synthesize a viscous polysaccharide material that forms the slime layer or bacterial capsule. |
| Proteolytic | *Bacillus subtilis, B. cereus,*<br>*Pseudomonas spp., Streptococcus sp.* | Degrade casein to peptides & amino acids. Pseudomonas may produce coloration of milk. |
| Lipolytic | *Pseudomonas fluorescens.*<br>*Achromobacter lipolyticum.*<br>*Candida lipolytica, Penicillium spp.* | Hydrolyze milk fat into glycerol & fatty acids. Some fatty acid impart rancid odor to the milk. |

Every Raw milk samples (RM) were selected for pasteurization and derived as Home Pasteurized samples (HPM) .Then ALP analysis was applied in two different procedure to verify ALP values of Raw milk samples (RM), Home Pasteurized samples (HPM) and UHT samples.

### 2.3.4 Phosphatase test by Conventional Colorimetric method

In titration method equipments should be required test tubes, 5 ml pipettes, 1 ml pipettes, l00 ml volumetric flask, 500 ml volumetric flask, water bath at 37ºC. All glassware must be rinsed, cleaned, rinsed in chromic acid solution and boiled in water for 30 min. Reagent should be prepared into two categories

Buffer solution: Is mixed by 0.75g anhydrous sodium carbonate and l.75g Sodium bicarbonate in 500 ml distilled water.

Buffer-substrate solution: Place 0.l5 g of disodium Para nitro phenyl phosphate / P-Nitro phenyl phosphate disodium salt (C6H4NO6PNa2.6H2O, The substrate) into a clean 100ml measuring cylinder.

Add the buffer solution to make to 100 ml mark. Store this buffer-substrate solution in a refrigerator and protected against light. It should not be used after one week. Prepare a fresh stock. Pipette 5mls buffer-substrate solution into a test tube, stopper and warm the solution in the water bath at 37ºC. Add to the test tube 1ml of the milk to be tested, stopper and mix well and place in water bath at 37ºC. Prepare a blank sample from boiled milk of the same type as that undergoing the test. Incubate both the test samples and the blank sample at 37ºC for 2hrs. After incubation, remove the tubes and mix them thoroughly. Place one sample against the blank in a Lovibond comparator using A.P.T.W. disc and rotate the disc until the color of the test sample is matched and read the disc number. The Phosphatase Test for Pasteurized Milk.

Disc Reading after incubation at 37ºC from 2 hrs then remarkable results can be determined by following range-

| | |
|---|---|
| Properly pasteurized | 0 to 10µl |
| Slightly under pasteurized | 10 to 18µl |
| Under pasteurized | 18 to 42µl |
| Not pasteurized | more than 42µl |

### 2.3.5 Phosphatase test by Optimized Standard Method

In the presence of magnesium and zinc ions p-Nitro phenyl phosphate is hydrolyzed by Phosphatase to form phosphate and p-Nitro phenol. The p-Nitro phenol released is proportional to the Alkaline Phosphatase activity and can be measured photometrical to determine the alkaline phosphates level presence in the milk. Colorimetric assay in accordance with a standard method followed by samples and then addition of Buffer solution (R1) and addition of buffer/substrate (R2).

$$\text{p-Nitro phenyl phosphate} + H_2O \xrightarrow{\text{Alkaline phosphatase}} \text{phosphate} + \text{p-Nitro phenol}$$

**Manual procedure for substrate start**

| | |
|---|---|
| Wave length | Hg 405 nm (400-420nm) |
| Temperature | +20$^0$C/ +30$^0$C/ +37$^0$C |
| Cuvette | 1cm light path |
| Zero adjustment | air or distilled water |

**Table 4**

| Working agent | Macro | Semi | Micro |
|---|---|---|---|
| R1 | 2500µl | 1000µl | 500µl |
| Sample | 50µl | 20µl | 10µl |
| R2 | 500µl | 200µl | 100µl |


**Figure 2:** Reagent (R2)


**Figure 3:** Buffer solution (R1) and Reagent (R2)

Clinical DGKC method is applied on pasteurized milk to determine alkaline Phosphatase level within a short time.

**Reagent concentration: R1**
Di ethanol amine buffer 1.0 mol/ l ($P^H$ - 9.8)
Magnesium sulfates 0.6 m mol/ l
Detergents and Stabilizers >0.1%

**Reagent concentration: R2**
p-Nitro phenyl phosphate 2.0 m mol/l
Stabilized, liquid 5.0 g /dl

**Preparation**
Centrifuge pasteurized milk sample for containing precipitate components to separate watery portion before performing the assay.5 ml of R1 are mixed with 1 ml of R2 .Sample is measured by 1/10 ml of R2 and mixed with previous mixture of Reagents.

## 3. Results

**Raw Milk Samples (**Table5)

| Samples | Conventional Colorimetric method | Results | Optimized Standard Method | Results |
|---|---|---|---|---|
| RM1 | more than 42µl | Discernible color | 545 | Non pasteurized |
| RM2 | more than 42µl | Discernible color | 542 | Non pasteurized |
| RM3 | more than 42µl | Discernible color | 560 | Non pasteurized |
| RM4 | more than 42µl | Discernible color | 575 | Non pasteurized |
| RM6 | more than 42µl | Discernible color | 623 | Non pasteurized |
| RM7 | more than 42µl | Discernible color | 685 | Non pasteurized |
| RM9 | more than 42µl | Discernible color | 430 | Non pasteurized |
| RM12 | more than 42µl | Discernible color | 580 | Non pasteurized |
| RM16 | more than 42µl | Discernible color | 560 | Non pasteurized |
| RM17 | more than 42µl | Discernible color | 620 | Non pasteurized |
| RM18 | more than 42µl | Discernible color | 476 | Non pasteurized |
| RM20 | more than 42µl | Discernible color | 530 | Non pasteurized |
| RM21 | more than 42µl | Discernible color | 457 | Non pasteurized |
| RM22 | more than 42µl | Discernible color | 278 | Non pasteurized |
| RM23 | more than 42µl | Discernible color | 567 | Non pasteurized |
| RM25 | more than 42µl | Discernible color | 450 | Non pasteurized |
| RM26 | more than 42µl | Discernible color | 680 | Non pasteurized |
| RM27 | more than 42µl | Discernible color | 372 | Non pasteurized |
| RM29 | more than 42µl | Discernible color | 565 | Non pasteurized |
| RM31 | more than 42µl | Discernible color | 542 | Non pasteurized |
| RM32 | more than 42µl | Discernible color | 290 | Non pasteurized |
| RM34 | more than 42µl | Discernible color | 523 | Non pasteurized |
| RM35 | more than 42µl | Discernible color | 610 | Non pasteurized |
| RM37 | more than 42µl | Discernible color | 624 | Non pasteurized |
| RM38 | more than 42µl | Discernible color | 578 | Non pasteurized |
| RM40 | more than 42µl | Discernible color | 348 | |

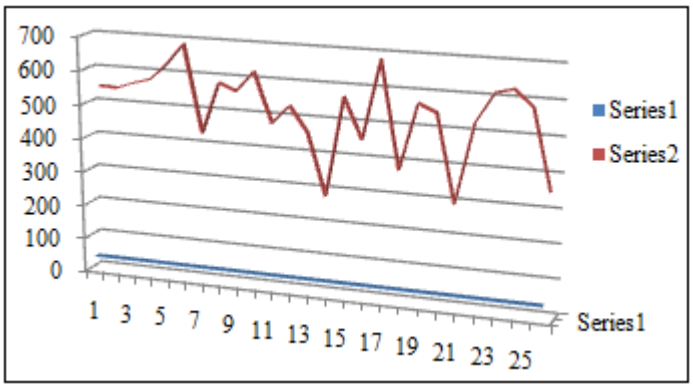



**Figure 1:** DGKC Method (Series2) shows high ALP values where as Conventional Method (Seres1) is parallel by a fixed value in highest range

**Home Pasteurized Samples: (**Table 6)

| Samples | Conventional Colorimetric method | Results | Optimized Standard Method | Results |
|---|---|---|---|---|
| HPM1 | 8 | No Discernible color | 15 | Moderate pasteurized |
| HPM2 | 10 | No Discernible color | 8 | Pasteurized |
| HPM3 | 5 | No Discernible color | 4 | Pasteurized |
| HPM4 | 7 | No Discernible color | 4 | Pasteurized |
| HPM6 | 5 | No Discernible color | 2 | Pasteurized |
| HPM7 | 4 | No Discernible color | 2 | Pasteurized |
| HPM9 | 12 | Discernible color | 16 | Moderate pasteurized |
| HPM12 | 6 | No Discernible color | 12 | Moderate pasteurized |
| HPM16 | 10 | No Discernible color | 8 | Pasteurized |
| HPM17 | 8 | No Discernible color | 6 | Pasteurized |
| HPM18 | 10 | No Discernible color | 10 | Moderate pasteurized |
| HPM20 | 14 | Discernible color | 23 | Slightly pasteurized |
| HPM21 | 10 | No Discernible color | 20 | Slightly pasteurized |
| HPM22 | 8 | No Discernible color | 6 | Pasteurized |
| HPM23 | 10 | No Discernible color | 8 | Pasteurized |
| HPM25 | 8 | Discernible color | 8 | Pasteurized |
| HPM26 | 10 | No Discernible color | 5 | Pasteurized |
| HPM27 | 8 | No Discernible color | 5 | Pasteurized |
| HPM29 | 10 | No Discernible color | 5 | Pasteurized |
| HPM31 | 5 | No Discernible color | 5 | Pasteurized |
| HPM32 | 5 | No Discernible color | 4 | Pasteurized |
| HPM34 | 2 | No Discernible color | 2 | Pasteurized |
| HPM35 | 8 | No Discernible color | 2 | Pasteurized |
| HPM37 | 7 | No Discernible color | 8 | Pasteurized |
| HPM38 | 7 | No Discernible color | 2 | Pasteurized |
| HPM40 | 10 | No Discernible color | 12 | Moderate pasteurized |

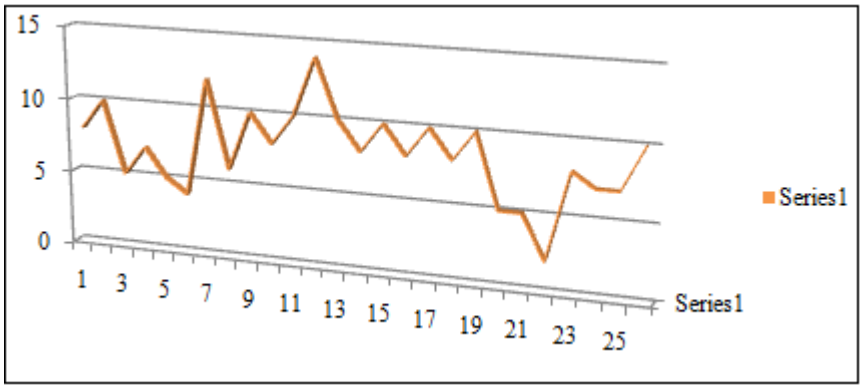



**Figure 1.1:** ALP values of HPM samples in Conventional Method (Seres1).

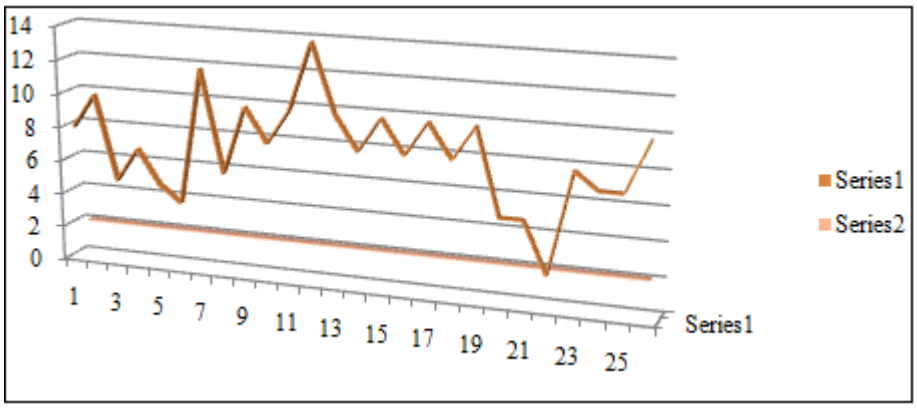



**Figure 1.2:** Fluctuation of ALP values in Conventional Method (Seres1) accordance with time 2hrs (Seres2)

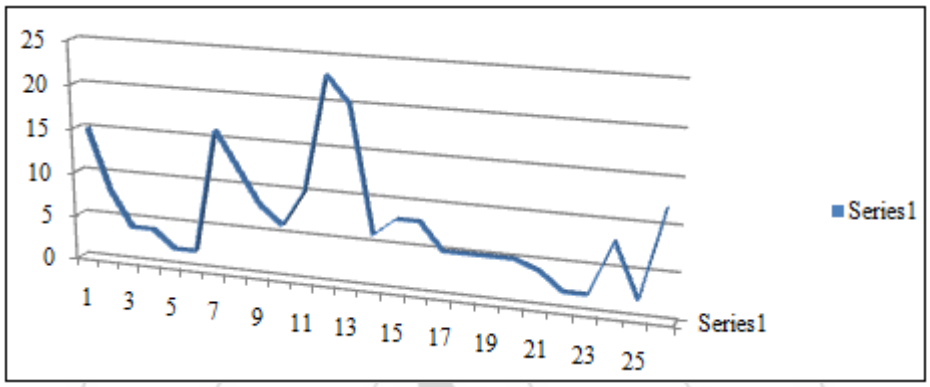



**Figure 1.3:** ALP values of HPM samples in Optimized Method (Seres1).

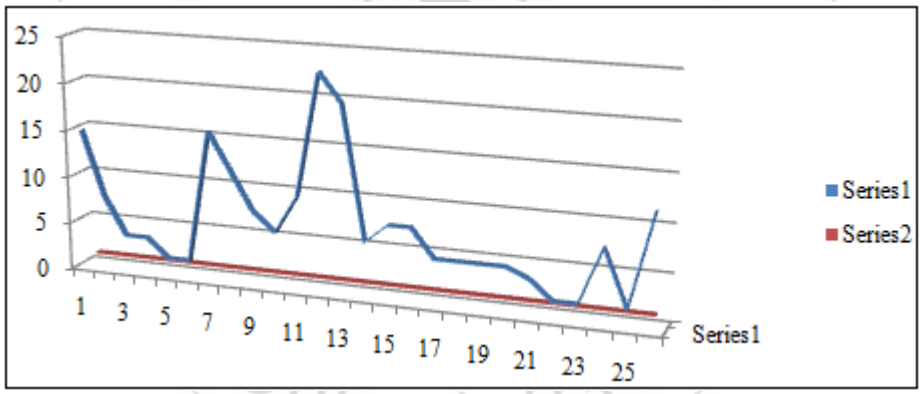



**Figure 1.4:** Fluctuation of ALP values in Optimized Method (Seres1) accordance with time 1hrs (Seres2)

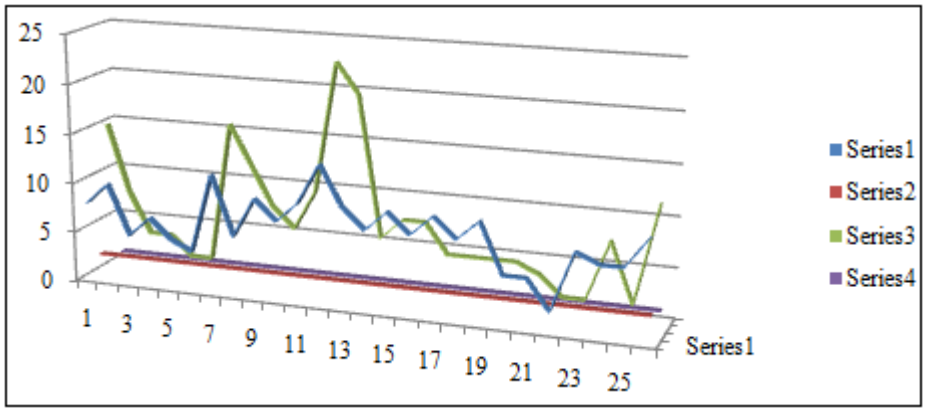



**Figure 1.5:** Comparative study of parameter (ALP values) in Conventional Method (Seres1) accordance with time 2hrs (Seres2) and Optimized Method (Seres3) accordance with time 1hrs (Seres4).

**UHT Samples: (**Table7)

| Samples | Conventional Colorimetric method | Results | Optimized Standard Method | Results |
|---|---|---|---|---|
| UHT1 | 8 | No Discernible color | 2 | Pasteurized |
| UHT2 | 8 | No Discernible color | 5 | Pasteurized |
| UHT3 | 5 | No Discernible color | 5 | Pasteurized |
| UHT4 | 4 | No Discernible color | 2 | Pasteurized |
| UHT5 | 5 | No Discernible color | 2 | Pasteurized |

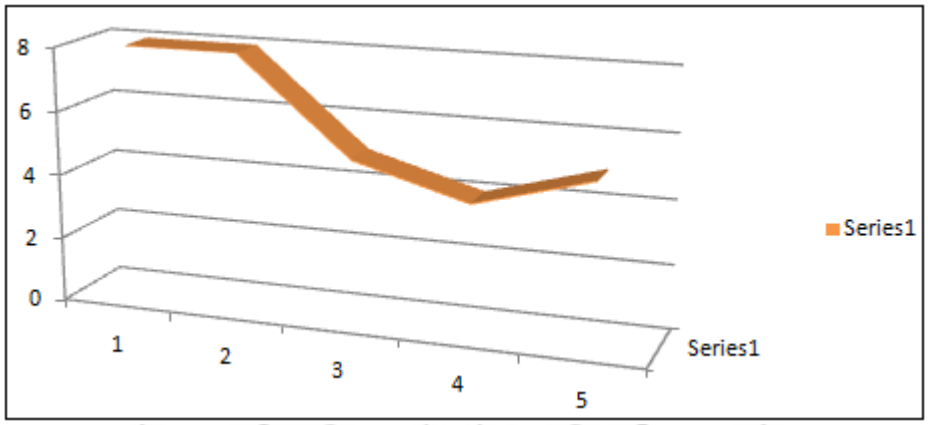



**Figure 1.6:** ALP values UHT samples in Conventional Method (Seres1).

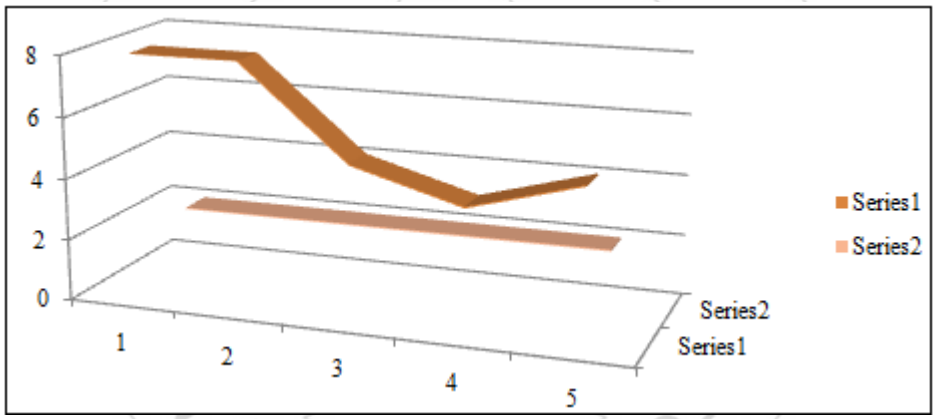



**Figure 1.7:** Fluctuation of ALP values in Conventional Method (Seres1) accordance with time 2hrs (Seres2)

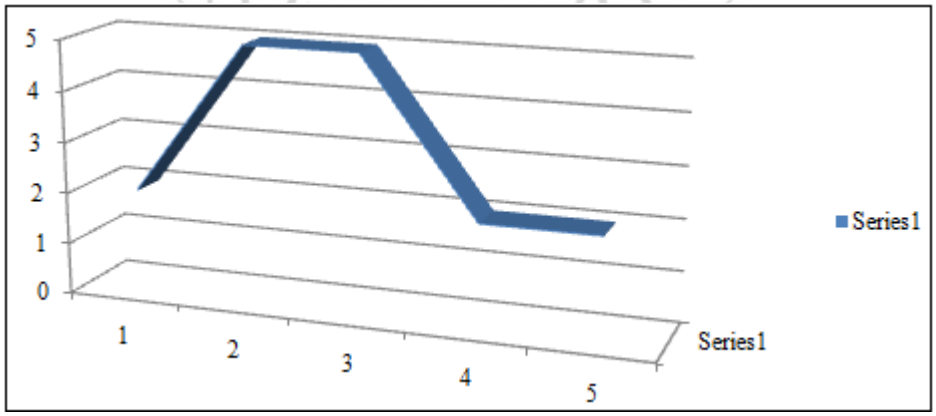



**Figure 1.8:** ALP values of UHT samples in Optimized Method (Seres1).

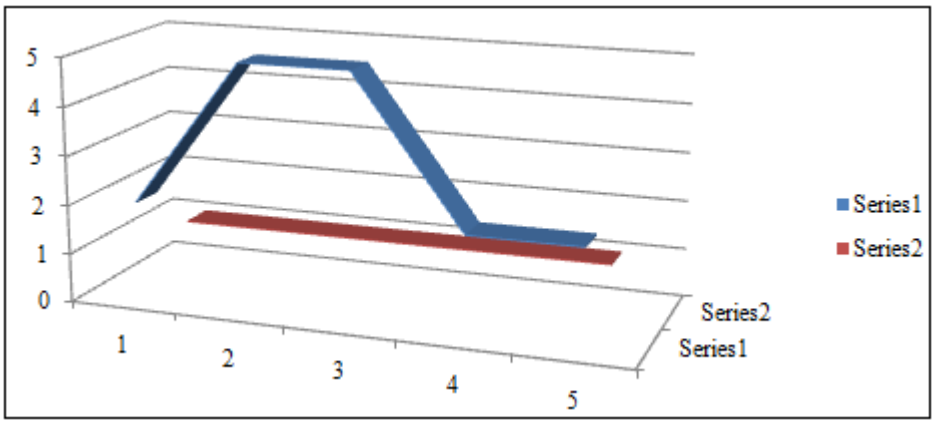



**Figure 1.9:** Fluctuation of ALP values in Optimized Method (Seres1) accordance with time 1hrs (Seres2)

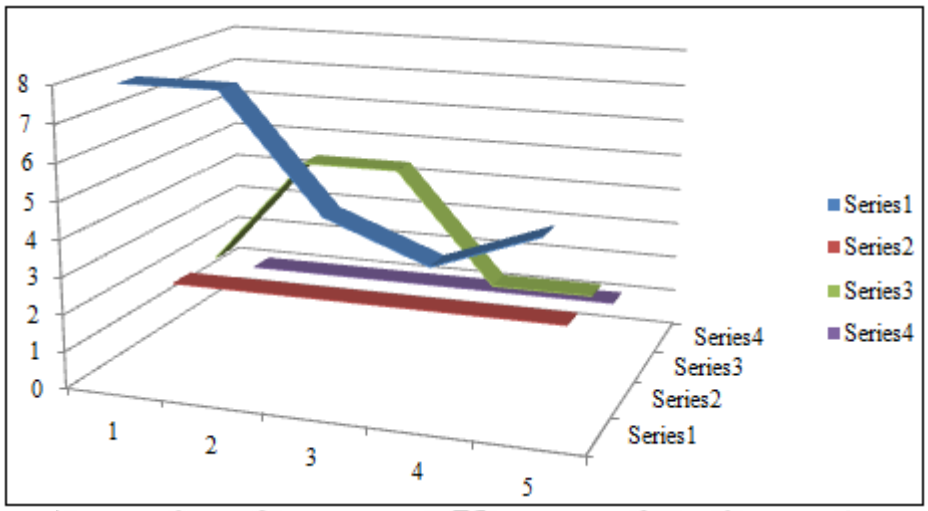



**Figure 2.1:** Comparative study of parameter (ALP values) in Conventional Method (Seres1) accordance with time 2hrs (Seres2) and Optimized Method (Seres3) accordance with time 1hrs (Seres4).

## 4. Discussion

Based on research findings, it should to discourage the consumption of raw milk. The risks of consuming raw milk instead of pasteurized milk are well established in the scientific literature, and in some cases can have severe or even fatal consequences. Then sample was pasteurized by home pasteurizer in our research laboratory. After pasteurization the proportion of raw milk samples (RM) was derived another samples, named Home Pasteurized Samples (HPM). DGKC Method (Series2) shows high ALP values where as Conventional Method (Seres1) is parallel by a fixed value in highest range of RM samples.

About discussing point of view in the table 5, RM samples resulted in the ALP that showing highest enzymatic degradation than table6 and table7 while future research could inform decision making on the legalization of raw milk. In between comparative study of table6 and table7 applied DGKC method is so easier procedure for perspective of ALP test. It was believed that from a public health perspective, it is a far safer choice to discourage the sale of raw milk along with Pasteurized and UHT milk without containing proper enzymatic reaction. Regardless, Milk borne harmful microbes may result the potential health risks of consuming Raw, Pasteurized and UHT milk should be clearly communicated, especially to vulnerable populations. In the beginning of research all collected samples were screened for determining the level of different values than applied for ALP analysis and graphical status showed that more fluctuation of ALP values resulted in Conventional Method (Seres1) accordance with time 2hrs (Seres2) in high level where as less fluctuation of ALP values occurred in Optimized Method (Seres1) accordance with time 1hrs (Seres2). These analytical parameter showed varifying pasteurization procedure resulted fluctuating milk components in between Conventional Method and Optimized Method. The potential benefits on the other hand, are still unclear and would benefit from further investigation. Here procedure left with a large uncertainty about the potential benefits of raw milk but with a clear understanding of the microbial hazards from consuming raw milk. So Clinical DGKC method is very much effective and accurate than Conventional method.

Raw milk

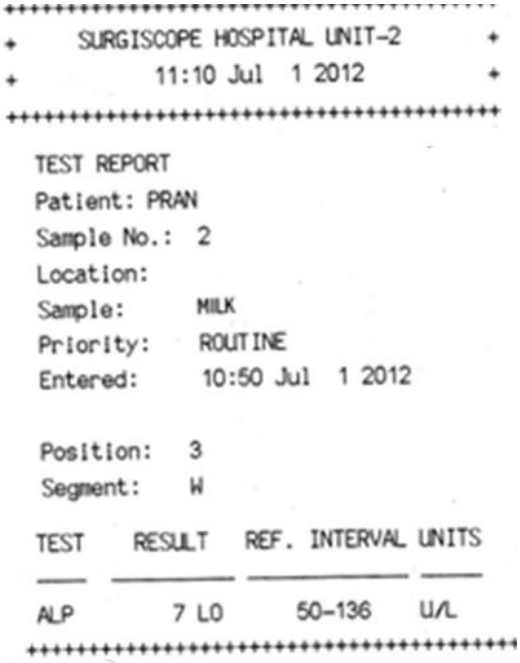
SURGISCOPE HOSPITAL UNIT-2
10:36 Jul 1 2012

TEST REPORT

Patient: UN P M
Sample No.: 2
Location:
Sample: MILK
Priority: ROUTINE
Entered: 10:19 Jul 1 2012

Position: 6
Segment: U

| TEST | RESULT | REF. INTERVAL | UNITS |
|---|---|---|---|
| ALP | 545 HI | 50-136 | U/L |



SURGISCOPE HOSPITAL UNIT-2
11:10 Jul 1 2012

TEST REPORT
Patient: PRAN
Sample No.: 2
Location:
Sample: MILK
Priority: ROUTINE
Entered: 10:50 Jul 1 2012

Position: 3
Segment: W

| TEST | RESULT | REF. INTERVAL | UNITS |
|---|---|---|---|
| ALP | 7 LO | 50-136 | U/L |

UHT

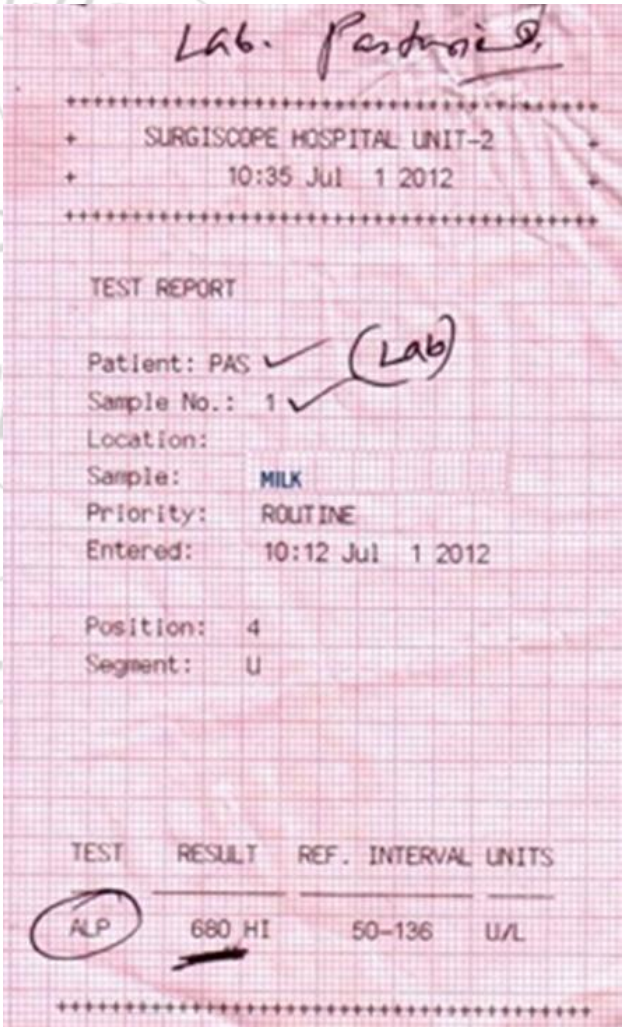
SURGISCOPE HOSPITAL UNIT-2
10:36 Jul 1 2012

TEST REPORT

Patient: UHT UN M
Sample No.: 3
Location:
Sample: MILK
Priority: ROUTINE
Entered: 10:13 Jul 1 2012

Position: 5
Segment: U

| TEST | RESULT | REF. INTERVAL | UNITS |
|---|---|---|---|
| ALP | 5 LO | abnl reaction | U/L |



Lab. Pasteurized

SURGISCOPE HOSPITAL UNIT-2
10:35 Jul 1 2012

TEST REPORT

Patient: PAS (Lab)
Sample No.: 1
Location:
Sample: MILK
Priority: ROUTINE
Entered: 10:12 Jul 1 2012

Position: 4
Segment: U

| TEST | RESULT | REF. INTERVAL | UNITS |
|---|---|---|---|
| ALP | 680 HI | 50-136 | U/L |

```
++++++++++++++++++++++++++++++++++++++++
+     SURGISCOPE HOSPITAL UNIT-2       +
+          11:09 Jul  1 2012           +
++++++++++++++++++++++++++++++++++++++++
  TEST REPORT
  Patient: FARM FRESH
  Sample No.:  1
  Location:
  Sample:      MILK
  Priority:    ROUTINE
  Entered:     10:50 Jul  1 2012

  Position:   2
  Segment:    W

  TEST    RESULT    REF. INTERVAL UNITS
  ____  __________ _____________ _____

  ALP         5 LO  abnl reaction U/L
  ++++++++++++++++++++++++++++++++++++++++
++++++++++++++++++++++++++++++++++++++++
+     SURGISCOPE HOSPITAL UNIT-2       +
+          11:09 Jul  1 2012           +
++++++++++++++++++++++++++++++++++++++++
  TEST REPORT
  Patient: PASTURIZED
  Sample No.:  3
  Location:
  Sample:      MILK
  Priority:    ROUTINE
  Entered:     10:49 Jul  1 2012

  Position:   1
  Segment:    W

 TEST    RESULT    REF. INTERVAL UNITS
 ____  __________ _____________ _____

 ALP         2 LO  abnl reaction U/L
++++++++++++++++++++++++++++++++++++++++
```


**Figure 2.2:** Indication


**Figure 2.3:** Pasteurization of Raw milk.

**Figure 2.4:** Samples

**Experimental Procedure**

**Figure 2.5:** Synchronization of Samples

**Figure 2.1:** Pour on milk in Home Pasteurizer

**Figure 2.6:** Samples for ALP analysis

**Figure 2.7:** Samples setup for ALP analysis

## 5. Conclusion

Effective heat treatment of milk is essential to ensure the absence of pathogenic microorganisms and hence product safety for the shelf life of pasteurized milk. If the Alkaline Phosphatase originally present in Raw milk, then test negative following pasteurization, the analytical or production manager can be sure that the proper temperature and time control during processing has been used. Raw milk, Pasteurized milk and UHT milk must still test as Alkaline Phosphatase positive, otherwise these cannot be classified as such anymore. We may conclude that UHT pasteurized milk (Class-1) has a refrigerated shelf life of two to three weeks, whereas Home pasteurized milk (class-1,2) can last much longer when refrigerated, sometimes two to three months. When UHT treatment is combined with sterile handling and container technology (such as aseptic he proper packaging), it can even be stored unrefrigerated for 3-4 months. So in case of Research purpose Pasteurized milk from Home Pasteurizer and UHT milk of various types to identify Microbial loading under the ALP test. So DGKC Method is very much improved procedure than Conventional performance.

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