Bibliography

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Adviser: Marietta Q. Amatorio, DVM

Abstract

A total of 15 samples were collected from randomly selected stalls and grocery stores selling repacked milk powder at Baguio City Public Market and were examined to determine the microbial load and the presence of coliform organism.

The coliform bacteria isolated and their frequencies are: *Enterobacteraerogenes*, 33%; *Escherichia coli,* 47%; *Klebsiellapneumoniae, 47%;Proteus mirabilis,13%*. Further, Standard Plate Count results range from 1.6M-9.3M .The repacked milk samples have microbial load exceeding the maximum limit.

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Chapter No. I

INTRODUCTION

Background of the Study

Milk is considered as nature's most complete food because of its unique composition. It's fairly suitable proportion of protein, carbohydrates, vitamins and minerals and other substances enhance and supplement the nutrient in poor quality foods. However, the Philippine dairy industry remains in its infancy stage despite the effort of the government and private sectors to uplift the local milk production. It comprises only 2% of the total domestic supply. Thus to meet the local demand, the dairy industry heavily relies on importation. The consumption also varies according to the form of the product. By volume, milk powder accounted for 41% of the importation. These Imported dairy products are often sold as reprocessed and repacked goods. Over the year, milk consumption has been increasing. Based on food consumption surveys of the Food and Nutrition Research Institute (FNRI), per capita milk consumption averaged 8.7 kg/year for the period of 1958-1969. This figure increased to 12.6 kg in 1978 and 16.0 kg in 1982(PCARRD, 1991).

Powdered milk is also a common item in UN food aid supplies, fallout shelters, warehouses, and wherever fresh milk is not a viable option. It is widely used in many developing countries because of reduced transport and storage costs (reduced bulk and weight, no refrigerated vehicles). As with other dry foods, it is considered nonperishable,

And is favored by survivalists, hikers, and others require nonperishable, easy-to-prepare food (Anonymous, 2012).

Perhaps the most difficult problem in handling and processing dairy products is their highly perishable nature. There have been a number of food borne illnesses resulting from the ingestion of dairy products made with milk that was not properly pasteurized or was poorly handled causing post-processing contamination**.** In order to avoid excessive spoilage, many dairy products were developed as a result of attempts to prolong storage life. These include Milk powder, evaporated milk, condensed milk, and cheeses. All have prolonged storage life but still subject to contamination (Anonymous, 2011). Further, Brock, 1978 stated that Milk contains relative few bacteria when it leaves the udder of a healthy cow.Since milk is such a good growth medium, it is better to sterilize or pasteurize before drinking, because such bacteria rapidly multiply. Though bacteria can be retarded or killed during pasteurization, consumers should be aware of contacting disease brought by improper food processing that leads to microbial contamination that cause spoilage, unpleasant odors and tastes and a food poisoning outbreak in a community due to ingestion of the bacteria and or their toxins present in the food(Quinn,1994).

Importance of the Study

Powdered milk is a convenient form of nutritious milk that doesn't require refrigeration. It is easily reconstituted and also an easily transported and stored dairy ingredient. It is an economical source of dairy solids. They are required for the manufacture of infant formulae and recipes for baked goods where adding liquid would render the product too thin. Powdered milk is also widely used in various sweet and certain products such as milk candy, chocolates, caramel candy, pulvoron, pastillas, snacks, soup, ice-cream, ice crumble toppings, coffee creamer, sauces and etc. (Anonymous, 2011).

Downes*et al.,* 2001 stated that powdered milk must be considered sensitive products from a public health aspect because they are often consumed after reconstitution without additional heating. It is well established that dried milk can be a source of foodbourne illness because of contamination. Probably the two most significant sources of contamination are the dairy utensils or the milk-contact surfaces. Unaseptic packaging from workers hands, Equipments such as fillers used in handling and processing as well as storage of these products may also serves as possible source of contamination and pathogen. Normally the packaging material contributes very little to the total microbial load in the finished product. If the dairy utensils or the milk-contact surfaces are inadequately cleaned, sanitized and dried, bacteria may develop in large number. Under very poor conditions these sources may increase the bacterial content of milk by millions per millilitre. Undesirable bacteria from these sources include coliform bacteria, lactic Streptococci, and thermodurics, those which survives pasteurization. These bacteria grow

well in milk hence endanger its keeping quality (Frazier and Westhoff, 1988).

It is therefore important to determine the bacterial load of the product to evaluate the safetiness of the food for the consumer.

Objectives of the Study

The study was conducted to:

1. determine the microbial load in repacked milk powder, and
2. determine presence of coliform organism.

Place and Time of the Study

Fifteen samples were collected from three different stalls and two grocery stores selling repacked milk powder at Baguio City Public Market and were brought to the Microbiology Laboratory Room, College of Veterinary Medicine, Benguet State University, La Trinidad, and Benguet for processing from August 2011 to March 2012.

Chapter No. II

REVIEW OF LITERATURE

Microorganisms are associated with most of the food we eat. These microorganisms can cause spoilage and lend up unpleasant odours and flavours to the milk. Also, milk can harbour pathogenic microorganism which produces disease if microbial populations are permitted to proliferate extensively. Certain microorganism produces toxic substances which results in food poisoning outbreak (McCuen*et al*., 1988).

Davis, 1962 reported that Bacterial standards for milk varies from place to place, some areas require that milk must contain not over 100,000 bacteria per ml, however, United State public health suggested a certain regulation of not over 200,000 bacteria per ml. Federal regulation defined in the pasteurized milk ordinance mandate that milk standard plate count should not be greater than or equal to 10^4 .While in the Philippines, the Standard Microbial Plate Counts shall not exceed 50,000 cfu/ml and coliform count should be less than or equal to ten cfu/ml (Anonymous,2007).

Wholesome, high quality milk and hygienic control of milk is indeed necessary, these involves many carefully controlled steps, regardless of whether the end result is filled milk or powdered milk. These hygienic controls involves such matter as clean milking operation and equipment, hygienic processing of the milk and proper storage and delivery to the end consumer. The genera comprising the coliform bacteria are principally *E.coli*, *Enterobacter* and *klebsiella*. Test for this bacteria are designed to measures the quality of the procedures for the handling and processing of the milk after the

pasteurization process (McCuen, 1988).

Coliforms are facultative anaerobes with an optimum growth at 37° C. Coliforms are indicator organisms; they are closely associated with the presence of pathogens but not necessarily pathogenic themselves. They also can cause rapid spoilage of milk because they are able to ferment lactose with the production of acid and gas, and are able to degrade milk proteins. Mabesa*et al.*, 1989 as cited by Dulay, 2008 reported that Coliform group of microorganism has been used as index of the sanitary quality of foods and sometimes as an indicator of sanitary conditions existing in environment or kitchen during handling and processing.Further, Enumeration of these organisms in heat pasteurized foods can be used to assess the adequacy of heating process designed to inactivate bacteria and in assessment of overall quality of food and hygienic conditions present during food processing (Downes*et al.*, 2011).

Escherichia coli is a natural inhabitant of the large intestine and small intestine of all mammals(Quinn *et al.*,1994).It causes coliform mastitis in cow and sow In dairy cows, the source of infection is faecal contamination of the skin of the mammary gland and relaxation of the teat sphincter following milking increases vulnerability to infection. In man it produces fatal intestinal infections in infants.*E.coli* is excreted in the faeces and can survive in faecal particles, dust and water for week or months. Because of its prominence as a normal intestinal bacterium in most mammals, most strains of *E.coli* are easy to detect in food and water and currently one of the indicator bacteria to monitor in water, foods and dairy products (Nester, 2004).

Enterobacteraerogenes is very common normal faecal flora and can be isolated from wound. It causes coliform mastitis in cattle and occasionally part of the mastitis-detritus-agalactia (MMA) syndrome in sows (Quinn *et al.,* 1994).

Klebsiellapneumoniae is normally found in the normal flora of the mouth, skin and intestine. It is present in the environment, vegetation, soil and in faeces. It also causes urinary tract and wound infection, otitis, meningitis and pneumonia in man (Hyde, 1989).

Proteus mirabilis is found in faeces of mammals and environment. It causes urinary tract infections in man, dogs and horses. Also associated with otitis externa in dogs and diarrhoea in young mink and lambs, calves, goats and pups (Hyde, 1989).

Chapter No.III

MATERIALS AND METHOD

Materials

Materials used in the study were: 15 milk powder samples from different stalls and grocery stores in Baguio City Public Market, Trypticase Soy Broth(TSB),phosphate Buffer, Xylose Lysine Deoxycholate Agar(XLD), Triple Sugar Iron Agar, Simmon Citrate agar, Indole Broth, Methyl Red Broth,Vogues-Proskauer Broth, Ehrlich's Reagents, Methyl Red Indicator, Crystal Violet, Gram's Iodine ,Acetone Alcohol, Safranin, mineral oil, glass slides, microscope, sterilizing oven, autoclave, electric stoves, Quebec colony counter, bacteriological incubator, weighing balance, denatured alcohol, alcohol lamp, Erlen Meyer flasks, graduated cylinder, inoculating loops and needles beaker, petridishes ,pipettes, spatulas, stirring rods, test tubes, test tubes racks, test tube brush, wire baskets, distilled water, staining can, marking pen and masking tapes.

Methodology

Pre-experimental phase

Three stalls and two grocery stores selling repacked milk powder at Baguio CityPublic Market were identified as collection sites (Figure No.1). In each stall, one fourth kilogram of milk powder sample was collected in each collection time with five days interval until a total of 15 samples were completed.Each sample was properly labelled.The materials used in this study were autoclaved and sterilized.

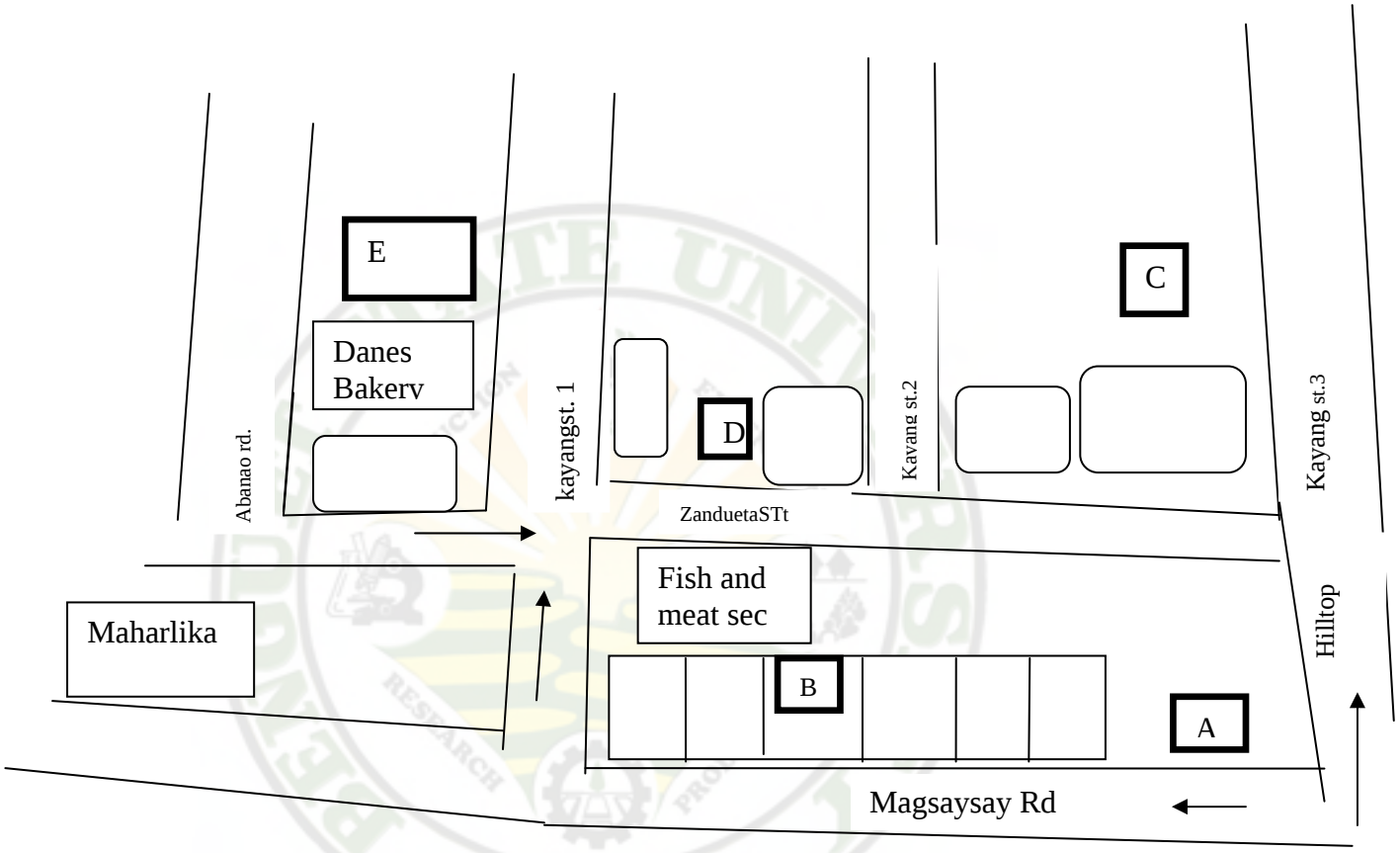


Figure No.1 Schematic Diagram of the sites of Collection of Samples
*A,B,C,D,E-Sites of collection

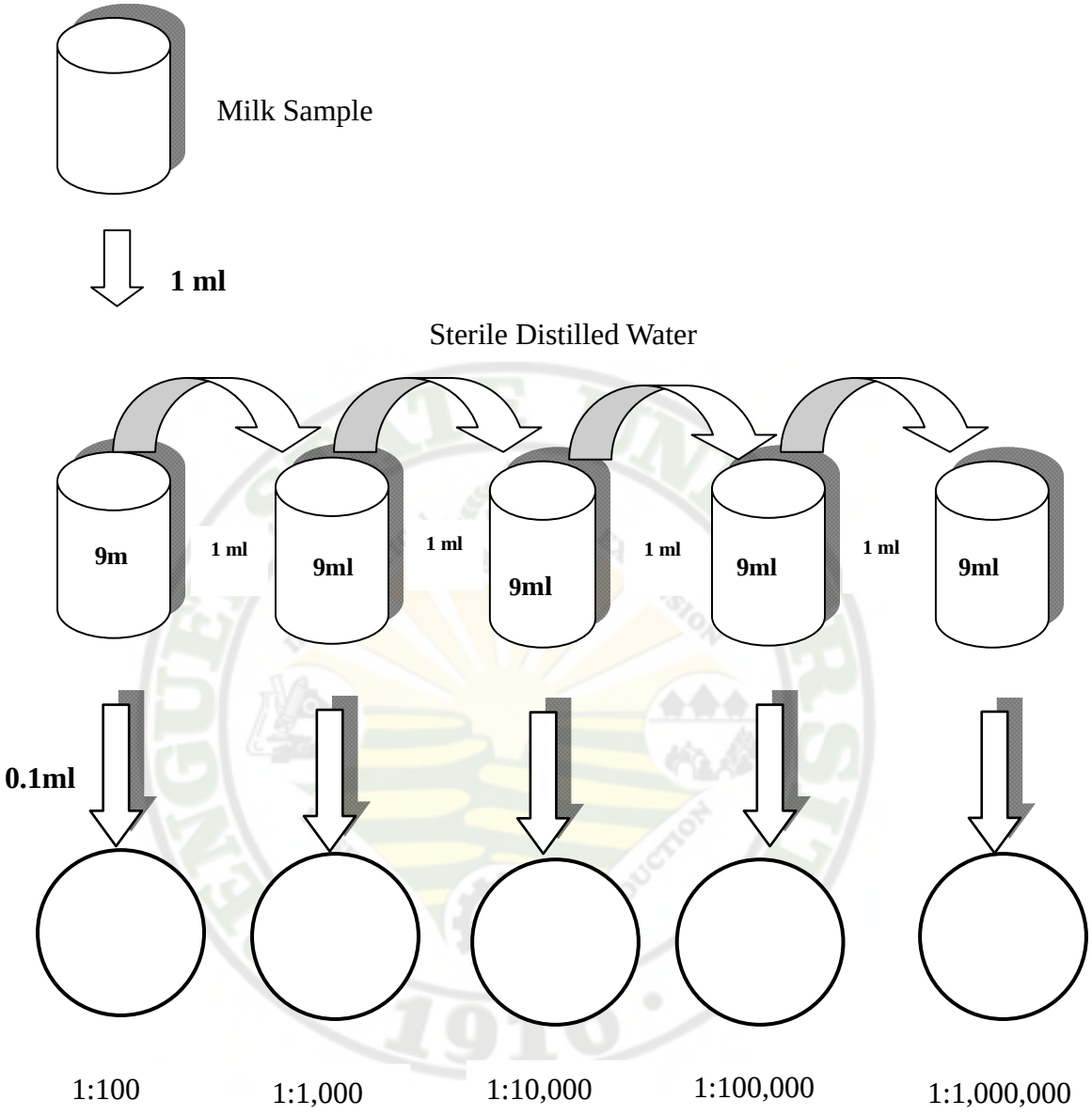
The media were prepared based on the manufacture's specification(Appendix D).

Experimental Phase:

Six grams of each sample in powdered form was reconstituted in 10millilitres (ml) phosphate buffered- distilled water at PH of 7.2. Using a pipette, one ml of the reconstituted milk powder was poured in Trypticase Soy Broth that served as cultivation medium for isolating bacteria and was incubated for 24 hours at 37^{o}C.

Standard Plate Count (Figure No.2)

A series of dilution was made on a five sterile test tubes containing nine ml of distilled water as diluents. One ml from the incubated sample in TSB was delivered to test tube one containing nine ml of sterile distilled water thereby giving a dilution of 1:100.It was mixed by swirling. Another one ml from test tube one was pipetted and delivered to test tube two containing nine ml distilled water giving a dilution of 1,000.It was then mixed by swirling. From test tube two, one ml was transferred to test tube three containing nine ml of distilled water giving a 1:10,000 dilutions. The same procedure was repeated to test tube four and five with a dilution of 1:100, 000, and 1:1,000,000 respectively.0.1 ml from each test tube was delivered to a sterile plate containing 20ml of melted nutrient agar tempered at 43-45 ^{0}C. It was gently swirled to mix and then allowed to cool and solidify. It was placed in an upside down position inside the incubator at 37c for 24 hours. The plate was examined for visible microbial growth. Counts were made from each plate using a Quebec colony counter. The average count from all plates were calculated and recorded to obtain the microbial count per ml of sample.



*****Number of bacteria per ml= number of colonies x dilution of sample/number of plates used.

Figure no.2 Standard Plate Count Method

Test for the Presence of Coliform Organism (Figure No.3)

A loopful of samples from TSB was inoculated to XLD and was incubatedat 37^0C for 24 hours. Colony from XLD suggestive of coliform was touch using an inoculating needle then was streaked to triple sugar iron and incubated at 37OC for 16 hours. This was later subjected to gram staining and to biochemical tests such as Indole test, methyl red test, vogues-proskauer test, citrate utilization test.

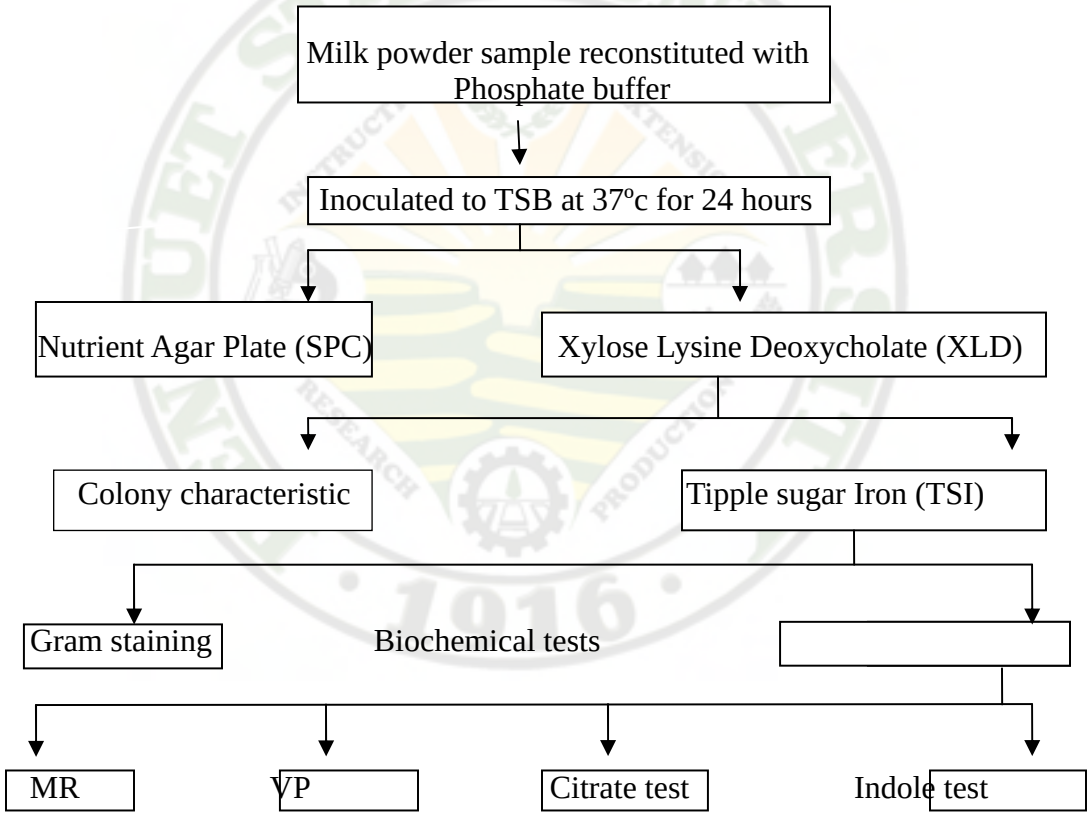


Figure no.3 Schematic Diagram for the Bacterial Isolation in Milk Powder

Data Gathered

The Data gathered were the following:

1. Source of samples: Milk powder samples were taken from different stalls selling Repacked Milk Powder at Baguio city public market (Figure no.1)

2. Mean Number of Bacteria Per ml of the Sample. This was computed by multiplying the colonies counted on each individual plate by their corresponding dilutions. The mean total number of the colonies will be divided by the number of plate used where the count will be made.

3. Colony Characteristic of Coliform Bacteria. The growth of coliform bacteria was observed according to their characteristics appearance on XLD (Appendix A).

4. Biochemical Tests. These were based on their reaction in MR, VP, and Indole And Citrate (Appendix A).

5. Identity of coliform organism. This was based on the colony characteristics on XLD agar, gram staining reaction and biochemical test (Appendix B).

Chapter No.1V

RESULTS AND DISCUSSION

Table No. 1 shows the mean number of bacteria per ml of repacked milk powder sold at Baguio City Public Market

TABLE No.1 Mean number of microorganisms per ml of sample, n=15

Sample No.	Mean count ($1x10^7$)
1	3.9
2	2.3
3	7.9
4	4.2
5	2.3
6	8.4
7	1.8
8	2.2
9	2.7
10	1.6
11	5.4
12	3.6
13	9.3
14	6.5
15	8.3

It shows that the Mean Number of bacteria per ml of the sample ranged from 1.6 x 10^7 to 9.3 x10^7.This indicates that all the samples are contaminated and have exceeded the maximum count of United State Public Health Services which is 200,000 per ml (Davis,1962) and the Philippines standard which is 50,000 cfu/ml of milk sample (Anonymous, 2007).

The very high load of Microorganism in the sample can be attributed to the improper repacking and storage of the product .It was observed that the samples were not properly sealed and the packaging materials were dusty and too vague. Samples were even stored in places accessible to pest infestation such as mice and fliesand arefreely exposed to air contaminants and passersby. Downes, 2011, reported that improper processing and unaseptic packaging lead to contamination. Further, Frazier and Westhoff, 1988 stated that very poor condition of the environment may increase bacterial count of milk by millions per ml.

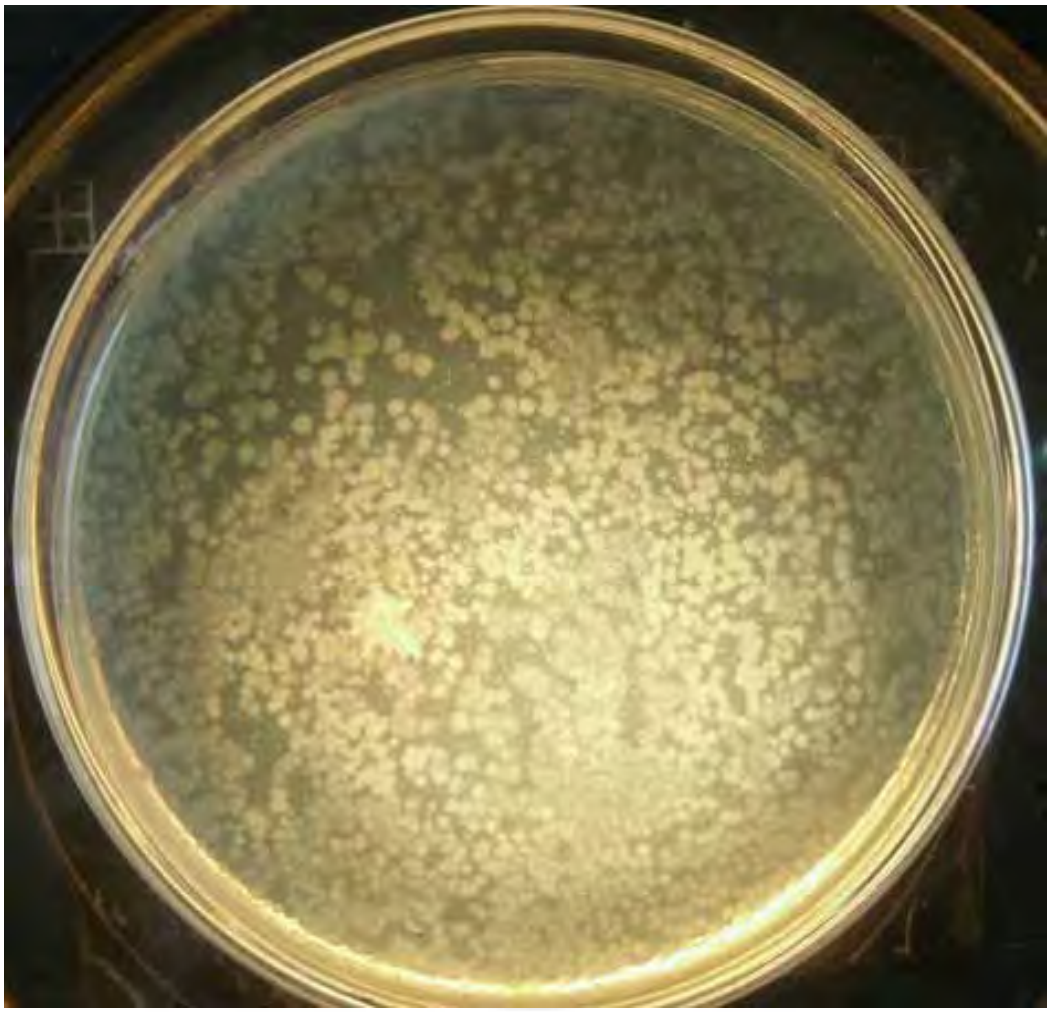


Plate No.1. Microbial Growth in Standard Plate Count at 1x 10^6 Dilutions

Table No.2 shows the identity of the coliform bacteria isolated from repacked milk powder.

Table No.2 Identity and frequency of coliform Organism in repacked milk powder, n=15

Identity of Micro-Organism	Gram stain	XLD colony	TSI	Indole	MR	VP	Citrate	Frequency (%)
E.aerogenes	gram Negative	yellow, mucoid and glistening	Y/Y	+	-	+	+	33%
E.coli	gram Negative	yellow, large flat, smooth and glistening	Y/Y, CO2	+	+	-	-	47%
K.pneumonia	gram Negative	yellow, mucoid, large and Smooth	Y/Y	-	+/-	+/-	+	47%
P.mirabilis	gram Negative	yellow with black centre	R/Y, H2S	-	+	-/+	+/-	13%

It shows that the Coliforms isolated and their frequencies are:*Enterobacteraerogenes*, 33%; *E.coli*, 47%; *Klebsiella pneumonia*, 47%.And *Proteus mirabilis*, 13%.

These coliform organisms thrive in the environment and in faecal particles. It was

mentioned earlier the condition of the samples and their storage.M
reportedthat these organisms are normal flora of faeces. Presence of it in v
indicates faecal contamination. All coliforms are killed by pasteurization. I
samplepost pasteurization can be attributed to improper handling, from equipments used in handling milk, from workers hands, and during storage of the product as mentioned by McCuen, 1988 and Frazier and Westhoff, 1988. Further, Pelczar, 1986 mentioned that foods are always subject to growth of any organism considering the method by which it is handled during processing.

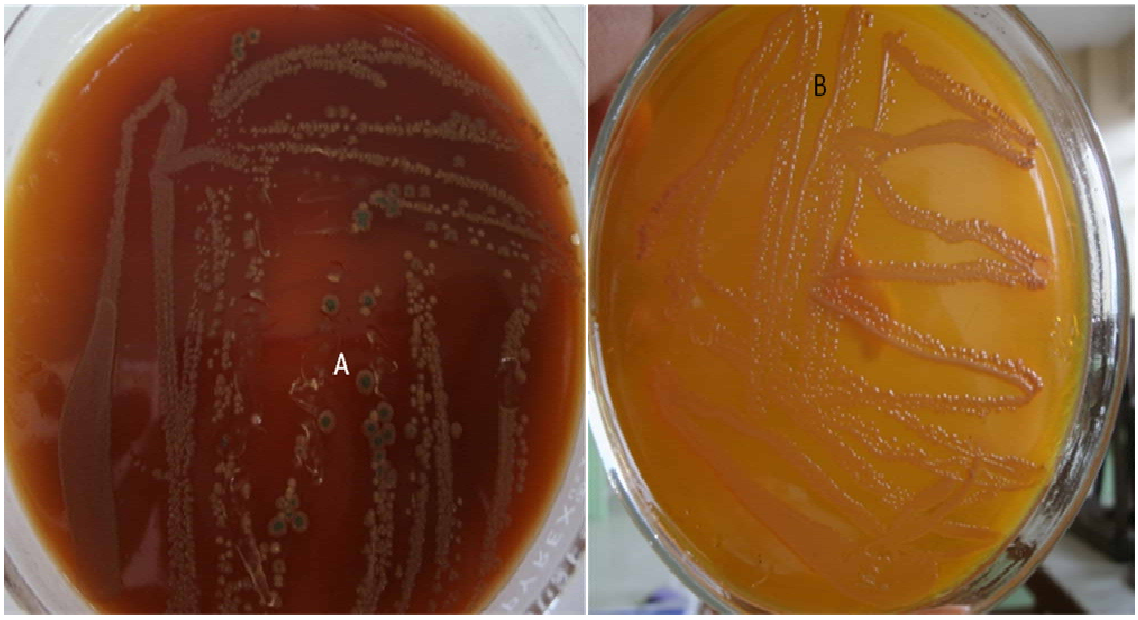


Plate no.2. Growth of coliform bacteria in XLD (A) colony with black centre and (B) Yellow colony

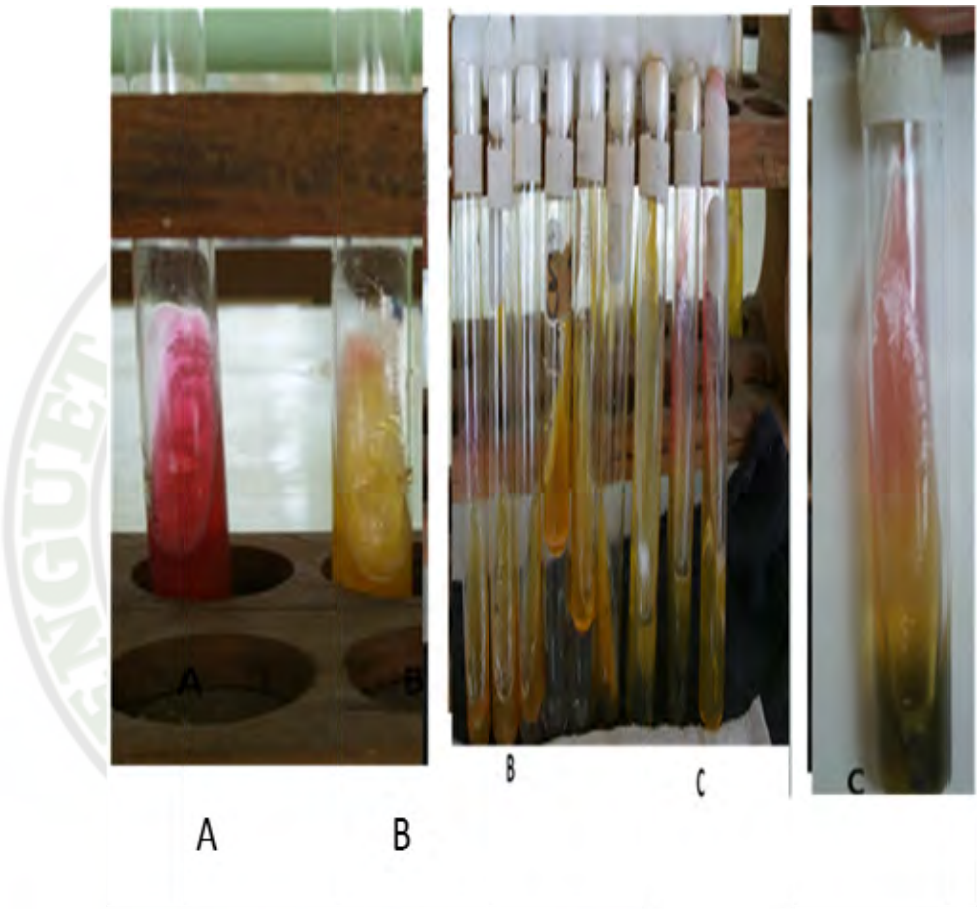


Plate no.3.Triple Sugar Iron Test. A.R/R (non-coliform) B.Y/Y (typical coliform) C. R/Y/H2S (*P.mirabilis*)

Plate no.4.Voges-Proskauer Tests A (+) and B (-)

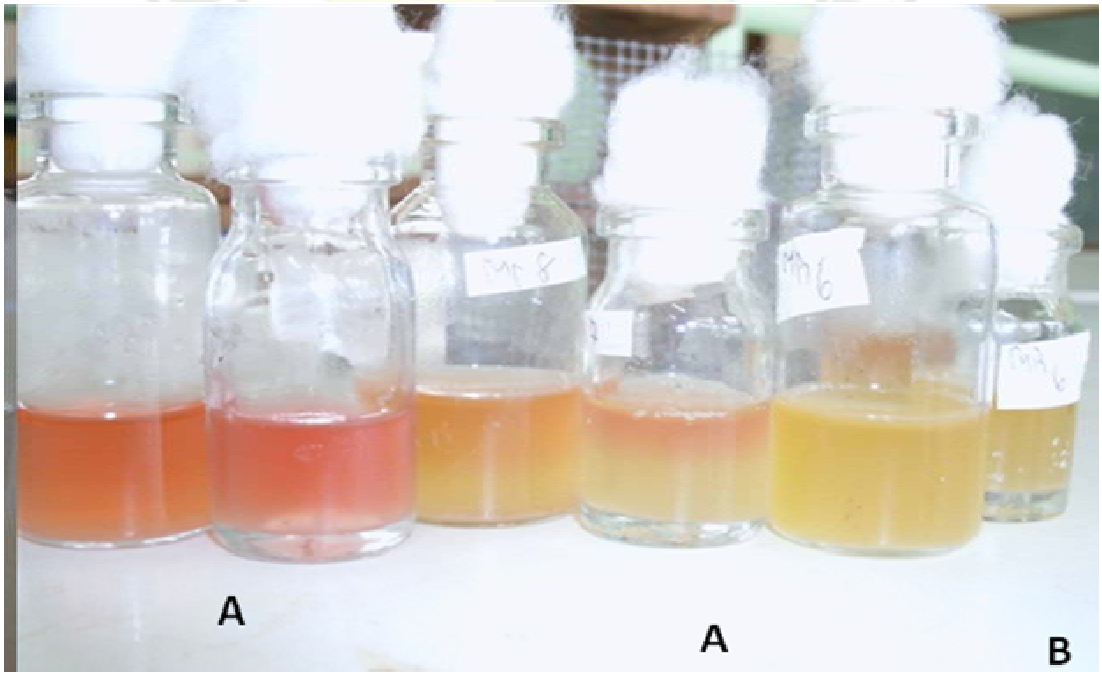


Plate No.5 Methyl Red Tests A (+) and B (-)

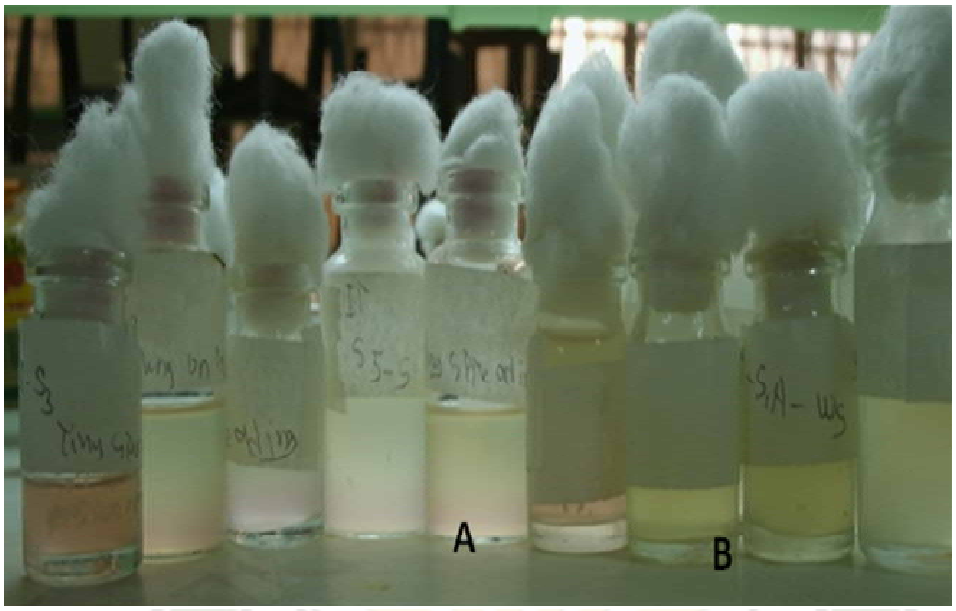


Plate No.6. Indole Tests A (+) and B (-)

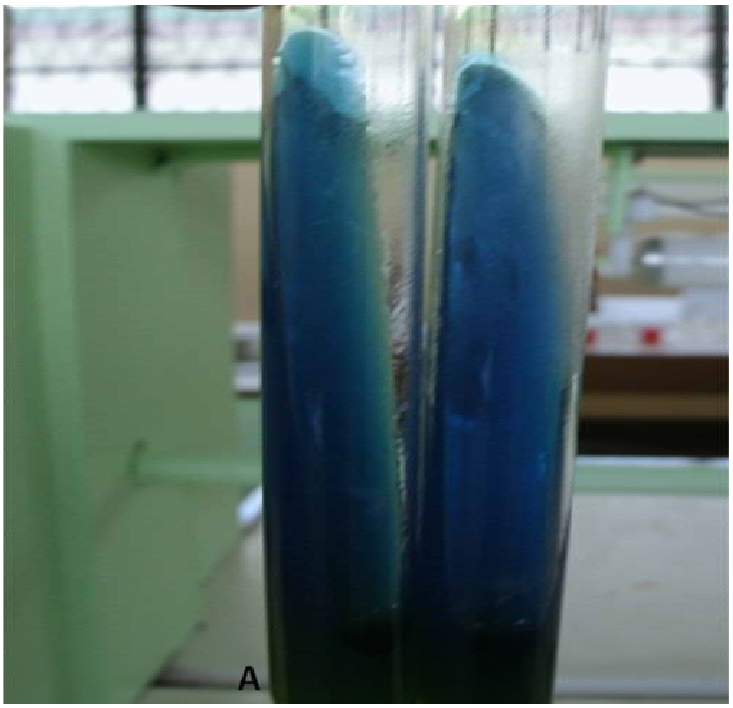


Plate no.7.Simmon Citrate Test A (+)

Chapter No. V

SUMMARY, CONCLUSION AND RECOMMENDATION

Fifteen samples were collected from randomly selected stalls and grocery stores selling repacked milk powder at Baguio City Public Market to determine the microbial load and the presence of coliform organism in each sample. Each sample were reconstituted in phosphate buffer and then inoculated to TSB. One ml of each sample from TSB was inoculated to nutrient agar for standard plate count. Further, loopful of sample from TSB were inoculated to XLD. Growth suggestive of coliform was streaked to TSI agar. Then subjected to Gram staining and Biochemical tests.

Results showed that the isolated coliform and their frequency were: *Enterobacteraerogenes*, 37%; *Escherichia coli*, 47%; *Klebsiellapneumoniae, 47% and Proteus mirabilis,13%*.And the mean number of microorganism per ml of sample ranged from $1.6x10^7$-$9.3x10^7$.

It is concluded therefore that the repacked milk powder samples are heavily contaminated with faecal coliform.

It is recommended that repacked powdered milk should be studied further on the presence of Gram positive bacteria, molds, yeast, most especially *Staphylococcus Spp*and *Salmonella* where poisoning have been recorded.

Chapter No.VI

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Appendix A

Expected Colonial Characteristic in XLD, TSI and Biochemical Test of Coliform Organisms.

Identity	Colonial characteristic in XLD	TSI	Biochemical Tests			
			MR	VP	I	C
Escherichia coli	Yellow colony, flat, small, smooth and glistening, circular in outline	Y/Y w/ CO2	+	-	+	-
Enterobacteraerogenes	Yellow colony, spreading moist, medium in size and glistening	Y/Y	-	+	+	+
Klebsiellapneumoniae	Large mucoid, Yellow colony, raised, and entire in outline	Y/Y	+ (-)	+ (-)	-	+
Proteus mirabilis	Yellow colony with black centre	R/Y w/ H2S	+	- (+)	-	+ (-)

Appendix B

Morphological Characteristic, TSI and Biochemical Test Reactions and Identity of Coliform Bacteria

Sample	TSB	XLD	Gram stain	TSI	MR	VP	SC	I	Organism identified
1	turbid	Tiny translucent, raised, circular	Positive cocci	R/R					Non coliform
		Tiny translucent, raised, circular	Positive cocci	R/R					Non coliform
		Tiny translucent, raised, circular	Positive cocci	R/R					Non coliform
2	turbid	Yellow spreading moist, and glistening	negative rod	Y/Y	-	+	+	- (+)	*Enterobacter aerogenes*
		Yellow, mucoidlarge,raised, and entire in outline	Negative rod	Y/Y	+	+	+	-	*Klebseillapneumoniae*
3	turbid	Yellow,large,raised, round and entire in outline	Negative rod	Y/Y	+	+	+	-	*Klebsiellapneumoniae*
		Yellow, flat, smooth, glistening and circular in outline	Negative rod	Y/Y	+	-	-	+	*Escherichia coli*

		Yellow spreading moist, and glistening	Negative rod	Y/Y	-	+	+	+	*Enterobactera erogenes*
4	turbid	Yellow,large,raised, mcoid and entire in outline	Negative rod	Y/Y	+	+	+	-	*Klebseillapneumoniae*
		Yellow spreading moist, and glistening	Negative rod	Y/Y	-	+	+	+	*Enterobactera*
									27
5	turbid	Yellow, flat, smooth, glistening and circular in outline	Negative rod	Y/Y	+	-	-	+	*Escherichia coli*
6	turbid	Yellow colonies with black center	Negative rod	R/Y	+	+ (-)	+ (-)	-	*Proteus mirabilis*
		Tiny translucent, raised, circular	Positive cocci	R/R					*Non coliform*
7	turbid	Tiny translucent, raised, circular	Positive cocci	Y/Y					*Non coliform*
8	turbid	Yellow, flat, smooth, glistening and circular in outline	Negative rod	Y/Y	+	-	+ (-)	-	*Escherichia coli*
9	turbid	No growth							
		No growth							
		No growth							
10	turbid	Tiny translucent, raised, circular	Positive cocci	R/R					Non coliform
		Tiny translucent, raised, circular	Positive cocci	R/R					Non coliform
		Tiny translucent,	Positive	R/R					Non coliform

		raised, circular	cocci						
11.	turbid	Yellow,large,raised, mucoid and entire in outline	Negative rod	Y/Y	+	-	+	-	*Klebsiellapneumonia*
		Yellow,flat,smooth, glistening and circular in outline	Negative rod	Y/Y	+	-	-	+	*Escherichia coli*
12	turbid	Yellow,large,raised, mucoid and entire in outline	Negative rod	Y/Y	+	+	+	- (+)	*Klebsiellapneumoniae*
		Yellow, flat, smooth, glistening and circular in outline	Negative rod	Y/Y	+	- (+)	-	+	
13	turbid	Yellow spreading moist, and glistening	Negative rod	Y/Y	-	+	+	+	*Enterobacteraerogenes*
		Yellow,large,raised, mucoid and entire in outline	Negative rod	Y/Y	+	+ (-)	+	-	*Klebsiellapneumoniae*
14	turbid	Yellow, flat, smooth, glistening and circular in outline	Negative rod	Y/Y	+	+ (-)	-	+	*Escherichia coli*
		Yellow,large,raised, mucoid and entire in outline	Negative rod	Y/Y	+	+ (-)	- (+)	-	*Klebsiellapneumoniae*
		Yellow colonies with black centre due to h2s production	Negative rod	R/Y	+	+ (-)	+	-	*Proteus mirabilis*
15	turbid	Yellow, flat, smooth, glistening and circular in outline	Negative rod	Y/Y	+	-	-	+	*Escherichia coli*
		Yellow spreading	Negative	Y/Y	-	+	+	+	*Enterobactera*

		moist, and glistening	rod						*erogenes*

Appendix C

Gram Staining, TSI and Biochemical Test Performed

A. Gram staining

This is the differential colouring solution that distinguishes gram negative bacteria from gram positive organisms.

Procedure:

1. Put a little amount of the colony in a dry and clean glass slide.

2. Place a small drop of distilled water at the centre of the slide and spread it evenly using a sterile wire loop.

3. Air dry and heat fix on a flame and cool.

4. Flood with crystal violet for 2 minute then wash with running water.

5. Drop grams iodine and let it stand for 1 minute and then rinse with running water.

6. Apply grams acetone decolourizer for 10 seconds then rinse.

7. Counter stain with safranin for 30 seconds and then rinse

8. Dry the stained smear and examine under microscope.

B.Tripple sugar iron test

Isolated colony is touched with an inoculating needle. A tube of TSI agar is stab inoculated in the middle of the agar to within 5mm from the bottom of the tube. On the withdrawal of the straight wire, the entire slant is streaked (right to the top)

incubated at 37 for 16 hours.

C.INDOLE TEST

A loopful of the test organism will be inoculated into Indole broth for 24 hours.Ehrlich's reagent will be added. A positive result is indicated by a Pink/Red layer forming on top of the liquid.

D.METHYL RED TEST

Methyl broth will be inoculated with pure culture from the plate and incubated for 24-48 hour at 37 ^{0}C.Five drops of Methyl Red Indicator will be added to the broth after incubation. A change in colour from yellow to red indicates a positive result.

E.VOGUES-PROSKAUER TEST

Vogues-proskauer broth will be inoculated with pure culture from the plate and will be incubated at 37 ^{O}C for 48 hours. After incubation, 0.6ml of 5%Naphtol in absolute ethyl alcohol 0.2ml of 40% potassium hydroxide containing 2% creatine will be added. Shake well and keep the broth undisturbed for three to ten minutes. A positive result will appear as bright red to orange in colour.

F.CITRATE UTILIZATION TEST

A slant of Simmon's Citrate Agar is streaked with a pure culture stock and incubated for 24 to 48 hours at 37c.The PH indicator Bromothymol Blue, changes the slant from green to blue if the growth and utilization occurs. It is negative if

remains green and is positive if it turns blue.

Appendix D

Culture Media, Broth and Reagent Preparation

1. Phosphate buffer

Ingredients

Potassium dihydrogen phosphate	26.22g
Sodium carbonates	7.78

Final ph 7.2+/-0.1

Direction:

Prepare a stock solution by dissolving 34g in 1 L distilled water. Dispense and sterilize, if desired. Store under refrigeration. Prepare a working solution by adding 1.25 ml of the stock solution to distilled water and make up to 1 L. Dispense in bottles or other

appropriates quantities and sterilize by autoclaving at 121^{O}C for 15 minutes.

2. Trypticase soy broth

Ingredients:	Grams per litre
Pancreatic digest of casein	17.0g
Papaic digest of soybean meal	3.0
Sodium chloride	5.0
Dipotassium phosphate	2.5
Dextrose	2.5

Direction;

Suspend 30g of the powder in 1 litre of distilled water. Mixed thoroughly and warm gently until solution is completed. Dispense and sterilize by autoclaving at 121C for 15minutes.

3. Nutrient Agar

Standard formula

Ingredients;	**grams per litre**
Peptic digest of animal tissue	5
Beef extract	1.50
Yeast extract	1.50
Sodium chloride	5.00
Agar	15
Final ph at 25c	7.4+/-0.2

Direction:

Suspend 28 grams in 1000 ml distilled water. Heat to dissolve the medium

completely. Sterilize by autoclaving at 15 pounds pressure (121c) for fifteen minutes.

4. Xylose Lysine Deoxycholate Agar (XLD agar)

Standard formula

Ingredient	**Grams/Litre**
Yeast extract	3.0
L-lysine	5.0
Lactose	7.50
Sucrose	7.50
Xylose	3.50
NaCl	5.00
Na Deoxycholate	2.50
Na Thiosulphate	2.50
Ferric ammonium Citrate	0.80
Phenol Red	0.80
Agar	15.0
Final ph	7.4+/-0

Direction:

Suspend 56.68 grams in 1000 ml distilled water. Heat with frequent agitation until the medium boils. Do not autoclave or over heat. Transfer immediately to a w 50 ^{0}C. After cooling, pour into sterile plates. It is advisable not to prepare l which will require prolonged heating.

5. Triple sugar iron test

Ingredients:	Grams per litter
Peptic digest of animal tissue	10g
Pancreatic digest of casein (casein enzymatic hydreolysate)	10
Sodium chloride	5
Lactose	10
Dextrose	10
Ferrous ammonium sulphate	0.2
Sodium thiosulfate	0.2
Phenol red	O.O25

Agar	13.0

Direction:

Suspend 50.4 g of the powder in 1L of distilled water. Heat to boiling to dissolve the medium completely.mix well and distribute into test tubes. Sterilize by autoclaving at 121C for 15 minutes. Cool in slanted position such that deep butts (1 inch long) are formed.

6. METHYL RED-Vogues Proskauer Medium (buffered glucose broth)

Standard formula

Ingredients	gram/Litre
Buffered peptone	7.00
Dextrose	5.00
Dipotassium phosphate	5.00

DIRECTION:

Suspend 17.0 grams in 1000ml distilled water. Distribute in test tubes in10ml amounts and sterilize by autoclaving at 15 pounds pressure (121c) for 15 mir

6A.METHYL red reagents

Ingredients

Methyl red (Mallinckrodt)	0.1
Ethyl alcohol	300ml
Distilled water	200ml

DIRECTION;

Dissolve methyl red in alcohol before adding distilled water. Store in a colored bottle in a cool and dry place.

7. Vogues-proskauer (VP) Reagent A

INGREDIENTS:

A-naphtol (sigma chemical Co)	5g
Ethyl alcohol (absolute)	100ml

DIRECTION:

Dissolve a-Naphtol in a small amount of ethyl alcohol and bring the volume to 100ml in a volumetric flask or cylinder. The alcohol should be almost colourless. Store in a colored bottled and store in a dry place.

8. Vogues-proskauer (VP) reagent b

Ingredients

Potassium hydroxide (KOH)	40g
Distilled water	100ml

DIRECTION:

Weigh out the KOH very quickly, since it is hygroscopic and will become caustic when moist. Add less than 100ml water to the pellets in a flask in a cold water bath to prevent overheating. Bring the volume to 100ml in a volumetric flaks or cylinder. Store this reagent in the refrigerator in a polyethylene bottle or one that has been socially

treated for storage chemical.

9. Simmon's citrate agar

Standard formula

Ingredients	grams/liter
Magnesium sulphate	0.20
Ammonium dihydrogen phosphate	1.00
Dipotassium phosphate	1.00
Sodium citrate	2.00
Sodium chloride	5.00
Bromothymol blue	5.00
Agar	15.00
Final ph (at 25C)	6.8+/-0.2

DIRECTIONS:

Dissolve ingredients. Distribute to tubes or vials. Before medium solidifies, incline tubes to obtain 4-5 cm slant and 2-3cm butts. Sterilize by autoclaving at 15 pounds pressure (121c) for 15 minutes. Final ph, 6.8+0.2.

10. Indole test:

Ingredients;	gram
Pancreatic digest of casein	20.g
Disodium phosphate	2
Dextrose	1
Agar	1

Potassium nitrate 1

Direction

Suspend 254g of the powder in 1 L distilled water. Add 2 g of agar for use as a motility medium.mix thoroughly. Heat and boil for 1 minute until completely dissolved. Dispense in regular testubes, filling them half full. Sterilise by autoclaving at 118 to 121C for 15 minutes.

Kovacs reagent for indole test

Amyl alcohol	22.5 ml
P-dimethylaminobenzaldehyde	1.5g
Hcl	7.5ml

Ehrlich's reagent for Indole test

Ingredients

Paradimethylaminobenzaldehyde	2 grams
Ethyl alcohol	190ml
Hydrochloric acid	40ml

Directions:

Dissolve paradimethylaminobenzaldehyde in 190 ml of 95% ethyl

Then gently add hydrochloric acid to the moisture by pouring on the sides of

Volumetric flask.**Appendix E**

Program of Activities of Thesis Performance

Bacterial Isolation from Repacked Milk Powder Sold at Baguio City Public Market

Date	Activity
October 15, 2011	Purchase of Reagents and Chemicals. Barrow instruments and washes them. Sterilization of Glassware.

October 16, 2011	First collection of sample and preparation of TSB and phosphate buffer stock solution.
October 17, 2011	The sample will be reconstituted with phosphate buffer then inoculated to TSB.Incubate for 24 hours at 37 ^{O}C. Preparation of XLD.
October 18, 2011	Loopful of mixture from TSB will be streak d into XLD.
	Preparation of Nutrient Agar.1 ml from TSB will be mixed with nutrient agar for standard plate count.
	Preparation of TSI.
October 19, 2011	Reading and recording of results from XLD ad nutrient Agar.
	Growth Suggestive of coliform will be streaked to TSI
	Preparation of media for biochemical tests.
October 20, 2011	Reading and Recording of results from TSI.
	Gram staining
	Inoculation for biochemical test
	2nd collection of sample
October 21, 2011	reading of results from Biochemical test
	Preparation of TSB and Phosphate buffer
October 22, 2011	the sample will be reconstituted with phosphate buffer then inoculated to TSB.incubate for 24 hours at 37 ^{O}C. Preparation of XLD.
October 23, 2011	Loopful of mixture from TSB will be streaked int
	Preparation of Nutrient Agar.1 ml from TSB wi with nutrient agar for standard plate count.
	Preparation of TSI.
October 24, 2011	Reading and recording of results from XLD ad nutrient Agar.
	Growth Suggestive of coliform will be streaked to TSI
	Preparation of media for biochemical tests.
October 25, 2011	Reading and Recording of results from TSI.

	Gram staining
	Inoculation for biochemical tests
October 26, 2011	reading of results from Biochemical test
	Preparation of TSB and Phosphate buffer
	3rd collection of sample
October 27, 2011	the sample will be reconstituted with phosphate buffer then inoculated to TSB.Incubate for 24 hours at 3^{O}C.Preparation of XLD.
October 28, 2011	Loopful of mixture from TSB will be streaked into XLD.
	Preparation of Nutrient Agar.1 ml from TSB will be mix with nutrient agar for standard plate count.
	Preparation of TSI.
October 29, 2011	Reading and recording of results from XLD and nutrient Agar.
	Growth Suggestive of coliform will be streaked to TSI
	Preparation of Media for Biochemical tests.
October 30, 2011	Reading and Recording of Results from TSI.
	Gram staining
	Inoculation for Biochemical Test
October 31, 2011	Reading of Results from Biochemical tests

Appendix F1

Benguet State University
College of Veterinary Medicines
La Trinidad, Benguet

October 13, 2011

Joseph A. Dianso, DVM
Dean, College of Veterinary Medicine
La Trinidad, Benguet

Attn: **Marjury M. Tabon**
Laboratory technician

Sir:

I, FlorenceG.Puctiyao will be conducting my thesis entitled, **"Bacterial Isolation from Repacked Milk Sold at Baguio City Public Market"** for the period of October 2011 to January 2012. In this regard, I would like to request that I will be allowed to borrow the following materials and equipment needed for the performance of the study.

The materials and equipments needed are:

1.2 500 ml Erlenmeyer Flasks
2.5 50 ml Beakers
3.2 100ml beaker
4. 30 Testube
5.2 100 ml Graduated cylinder
6.1 50 ml graduated cylinder
7.20 Petri plates
8. 2 Inoculating loops
10.2 Inoculating needles
9. Pippetes
11.2 Testube brushes
12. 2 Test tube holder
13. 3 Test tube racks
14. 1 Alcohol lamp
15. 4 Wire baskets
16. 2 Stirring rod
17. 1 thermometer
18.2 spatulas
19.1 staining can

Rest assured that I will be held responsible and liable for any damage and lost of any of the said items.

Thank you very much and hoping for your consideration regarding this matter.

Respectfully yours,

Florence G.Puctiyao
Researcher

Noted:

Marrietta O.Amatorio,DVM
Thesis Adviser

Marjury M. Tabon
Laboratory technician

Approved by:

Joseph A.Dianso,DVM
College Dean

Appendix F2

Benguet State University
College of Veterinary Medicines
La Trinidad, Benguet

October 13, 2011

Joseph A. Dianso, DVM
Dean, College of Veterinary Medicine
La Trinidad, Benguet

Sir:

I, FlorenceG.Puctiyao will be conducting my thesis entitled, "**Bacterial Isolation from Repacked Milk Powder Sold at Baguio City Public Market**" for the period of October 2011 to January 2012. In line with this, I would like to request your good office for the use of the autoclave, water bath, refrigerator, weighing scale, Incubator, microscope and stove as well as the microbiology Room for the performance of the said thesis.

Rest assured of the proper care and use of the said items. Any untoward incidence during the said activity will be my sole responsibility.

Thank you very much and hoping for your favorable response.

Respectfully yours,

Florence G. Puctiyao
Researcher

Noted:

Marrietta O. Amatorio, DVM
Thesis Adviser

Approved:

Joseph A. Dianso, DVM
College Dean

Appendix G1

APPLICATION FOR ORAL DEFENSE

Benguet State University
COLLEGE OF VETERINARY MEDICINE
LaTrinidad, Benguet (2601)

APPLICATION FOR FINAL ORAL DEFENSE

Name: **PUCTIYAO, FLORENCE G.**
Student Number: 0608051

Thesis Title: **BACTERIAL ISOLATION FROM REPACKED MILK POWDER SOLD AT BAGUIO CITY MARKET**

Date and Time of Examination: February, 29 2012, 9:00 AM

Place of Examination: CVM Accreditation Room

MEMBERS OF THE EXAMINING COMMITTEE

Name of Member	Signature
CRISELDA S. BATTAD, DVM, MS	_____
JOSEPH A. DIANSO, DVM	_____
EDLYN MAE N. CIANO, DVM	_____

Recommending Approval:

MARIETTA Q.AMATORIO
Adviser

Approved:

JOSEPH A. DIANSO, DVM
Dean

Appendix G2
Benguet State University
COLLEGE OF VETERINARY MEDICINE
LaTrinidad, Benguet (2601)

REPORT OF RESULTS OF FINAL ORAL DEFENSE

Name: **PUCTIYAO, FLORENCE G.**
Student Number: 0608051

Thesis Title: **BACTERIAL ISOLATION FROM REPACKED MILK POWDER SOLD AT BAGUIO CITY MARKET**

Date of Examination: February, 29 2012

Place of Examination: CVM Accreditation Room

Name and Signature of Examinees: Remarks

MARIETTA Q.AMATORIO, DVM _____
Adviser Date

JOSEPH A. DIANSO DVM _____
Panel Date

EDLYN MAE NABUSANCIANO,DVM _____
Panel Date

CRISELDA SILVESTRE-BATTAD, DVM,MS _____
Department chairman Date

JOSEPH A. DIANSO DVM _____
Dean Date

BIOGRAPHICAL SKETCH

Name: **FLORENCE GOLWINGON PUCTIYAO**

Date of Birth: **October 05, 1989**

Home Address: **Lower Lubo, TanudanKalinga**

E-mail Address: **fp_akawilma@yahoo.com**

Education:

Elementary:	**Name of School:**	**Lubo Elementary school**
	Address:	**LuboTanudan, Kalinga**
	Year Graduated:	**2002**
Secondary:	**Name of School:**	**St.William's Academy**
	Address:	**Bulanao, Tabuk, Kalinga**
	Year Graduated:	**2006**
College:	**Name of School:**	**Benguet State University**
	Address:	**La Trinidad Benguet**
Degree and Year of Graduation:		**Doctor of Veterinary Medicine 2012**

Organization and Extra-curricular Activities:

Rodeo Club Philippines (Batch STEROIDS 2006)	**2010-2011: Whips**
	2011-2012: Examiner
BSU-Highland Cowboys/Cowgirls:	**2010-present**
Clinicians Club	**2010-2012**
Girl Scout of the Philippines	**2009-2010**