BASELINE ASSESSMENT OF THE MICROBIAL CONTAMINATION OF LORI CHEESE SOLD IN YEREVAN MARKETS

Master of Public Health Thesis Project Utilizing Professional Publication Framework

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ABSTRACT

This study focuses on microbial food contamination at the market level and answers the following research question: does Lori cheese, sold in open-air markets of Yerevan, conform to the food safety standards of the United States Department of Agriculture (USDA) and Armenia by the following parameters: total coliforms, *Escherichia coli* O157:H7, salmonella, *Listeria monocytogenes*, and molds that produce toxins. This was a comprehensive survey with cross-sectional analytical study design, which could be used as a baseline for future inspection efforts. The objectives of the program were:

1. Provide current data on the prevalence and levels of coliforms, Enterobacteriaceae, *Escherichia coli*O157:H7, salmonella, *Listeria monocytogenes*, and molds in each of 60 samples of Lori cheese sold in Yerevan open-air markets during September 2003.
2. Publicize the information from microbiological analysis to the public about significance of health and environmental risks in order to provide further actions toward minimizing.

From 60 analyzed samples of Lori cheese, collected from open-air markets of 12 Yerevan districts, in 85% of the samples the total coliform counts exceeded the USDA standard for safety dramatically: from 1 to more then 1,000 cfu/g in 1:10,000 dilution. For the members of the Enterobacteriaceae family and molds this numbers reached to 70% and 20% respectively. *E coli* O157:H7 and *Listeria monocytogenes* were found in 2 samples each and no salmonella species registered in any sample.

The findings of this study supported the hypothesis that Lori cheese had elevated microbial counts resulting from improper farming, milking, handling, and selling practices and is a threat to the public's health. These findings serve as a basis for recommending implementation of improved sanitary standards and monitoring of products sold in the markets of Yerevan.

1. INTRODUCTION

More than 200 known diseases are transmitted through food (1). The causes of foodborn illness include viruses, bacteria, parasites, toxins, metals, and prions, and the symptoms for foodborn illness range from mild gastroenteritis to life-threatening cancers and neurologic, hepatic, and renal syndromes (2). Safe foods are those that are free from contamination of toxic substances and microbial pathogens, which could result in disease (1,3). Knowing how to protect food from contamination is a managerial responsibility (4,5). The health and well being of a population, especially vulnerable groups, are closely related to the availability and safety of food (5). Periodic outbreaks of foodborn illnesses are common in Armenia. According to data of Ministry of Health, 209 cases of salmonellosis, 156 enteritis, and 397 cases of intestinal infection caused by unknown pathogens were registered during 2001 in Yerevan. During the same year, an outbreak of dysentery was registered in August. Many outbreaks and mild illnesses likely go unreported. But there have been few, if any, studies to confirm the extent of the problem of food contamination in Armenia (6).

Surveillance of foodborn illnesses is complicated by several factors (3,7,8). The first is underreporting. Milder cases are not detected unless foodborn illnesses are severe or even fatal. Second, many foodborn diseases are also transmitted through water or from person to person. And, finally, many pathogens of foodborn diseases have not yet been identified and cannot be diagnosed. However, for the most part, the sources of contamination are well-known microorganisms found in food of animal origin, including dairy products (9).

The Ministry of Agriculture, the State Veterinary Department, and the Ministry of Health's Republic's Center of Hygiene and Epidemiological Control, are responsible for food inspection (both locally produced and imported from other countries). According to Federal Legislation #13788533 (2002) all imported, exported, and locally distributed products of animal origin must be inspected for contamination and should satisfy the Republic Norms of

Quality (10). However, it is verified fact that food products in Yerevan markets do not adhere to hygienic norms (6). According to informal, farming, milking, treatment of diseased animals, and milk pasteurization as well as sanitary practices during transportation and marketing, inspections, and examinations for microbial contaminants of ready to eat products are often violated (11). Moreover, in recent years, there has been considerable interest in local private manufacturing production. Even though inspection of these products is regulated by federal legislation, often their production, transportation, distribution, and trading conditions are not adequately monitored (11).

The Republic of Armenia, as a country in transition, has faced different problems during the past 10 years. Food production and distribution processes have not worked properly since Soviet system collapse because of lack of governmental control. Underdeveloped infrastructure, inefficient distribution system and lack of proper storage facilities create significant difficulties in meeting both local and international food safety standards.

Because of severe economic difficulties people in Armenia have changed their food consumption preferences. However, for Armenians, cheese, particularly Lori cheese, is still one of the most popular national products that is affordable and available in many markets. The expectations of consumers are that the cheese they purchase is safe for consumption (2). Nevertheless, it was suspected that microbial contamination of cheese might be a problem because of the following: In Yerevan, public food markets provide unsanitary condition for food storage (moister and water content, temperature, and presence of microbial agents) and thus contribute to spoilage by the growth of pathogenic microorganisms and their toxins (12). At the same time not only sellers but also large number of people have access to the food through touching and selecting products, which causes additional contamination. Thus

markets fail to provide the level of product safety and sanitary condition of cheese sold in Yerevan.

Armenian national standards (GOST) require strict regulation norms (13) (Table 1). Comparison to international standards, particularly United States Department of Agriculture (USDA) standards, reveals that USDA standards are less strict in coliforms regulation and similar for other type of pathogens (Table 1). Thus it was interesting to see to what extent Lori cheese conforms to US and Armenian standards for soft cheeses (12).

Table 1. Microbiological Standards for Soft Cheese

Test	Standard in Regulations		Target
	GOST	USDA	
Total coliforms	Absence in 1ml 1:1,000 dilution	Absence in 1ml 1:10,000 dilution	Absence in 1ml 1:10,000 dilution
Enterobacteriaceae family members	Not specified in Regulations	Not specified in Regulations	Absence in 1ml 1:10,000 dilution
Listeria monocytogenes	Absent in 25g	Absent in 25g	Absent in 25g
Salmonella	Absent in 25g	Absent in 25g	Absent in 25g
Escherichia coli O157:H7	Not specified in Regulations	Not specified in Regulations	Absent in 25g

This was an exploratory study to identify the microbiological risk of Lori cheese sold in Yerevan markets. Although some studies have been performed on microbial contamination during the manufacturing of cheese, which revealed 80% of samples were contaminated with coliforms and members of Enterobacteriaceae family (12), no study have been conducted for Lori cheese sold in the markets.

Microbes are the most important causes of food contamination (3). According to scientific consensus and Food Safety and Inspection Service (FSIS) of USDA, total coliforms and generic E. coli as well as large group of microorganisms of Enterobacteriaceae family that includes salmonella species, are useful indicators for food sanitary control (14). Such analysis is relatively easy and inexpensive to perform, and the levels of the organism can be measured (14). Because among generic E. coli, *E. coli*O157:H7 is one of the most harmful to

human health and because in Armenia, this particular strain never was tested before (12), it was a matter of interest for this project. *Listeria monocytogenes* was included in the study because of the severe illnesses it causes and because it is also a good indicator of the sanitary condition of the milk products sold in markets (15). Among the important indicators of environmental sanitary condition of the food production, distribution, and marketing, is a mold growth. The following are the main characteristics of target microorganisms (detailed in Appendix 1).

Coliforms are a group of microorganisms that live in the guts of warm-blooded animals (16). Coliforms, specifically fecal coliforms, indicate the possible presence of fecal contamination from warm-blooded animals. These bacteria, though not generally pathogenic themselves, serve as indicators of the presence of organisms that may be pathogenic (16,17).

Enterobacteriaceae is a group of gram-negative bacteria encountered among the most pathogenic that can cause infections of the digestive tract or other organs of the body (17). They are the causative agents of such diseases as meningitis, bacillary dysentery, typhoid, and food poisoning. In most cases, the pathogenicity of a particular enteric bacterium can be determined by its ability to metabolize lactose. The group Enterobacteriaceae includes the bacteria Enterobacter, Escherichia, Klebsiella, Morganella, Proteus, Providencia, Salmonella, Serratia, Shigella, and Yersinia (17,18).

E. coli O157:H7 is one of hundreds of strains of the bacterium Escherichia coli. Although most strains are harmless and live in the intestines of healthy humans and animals, this strain produces a powerful toxin and can cause severe illness and infections not only in immunocompromised hosts but also in healthy humans (1,17,19). *Escherichia coli*O157:H7 is an emerging cause of foodborn illness. Infection often leads to bloody diarrhea and occasionally to kidney failure. Infection can occur after eating uncooked beef, drinking raw milk, and after swimming in or drinking sewage-contaminated water. Person-to-person

contact is also an important mode of transmission (20). Detection of it is used as a general indicator of the sanitary condition in the food-processing environment (1,19,21).

Salmonella species are among the most common bacterial causes of gastroenteritis in humans (17,18,21). Salmonellae are transmitted by a wide variety of agricultural products and processed foods as well as from animals to humans. Healthy adults are susceptible to infection, although illness tends to be more severe among the very young, the very old, or patients with underlying immunosuppression. Certain serotypes or serogroups are characteristically more virulent than others (1).

Listeria monocytogenes, the causative agent of the disease listeriosis, has emerged as a foodborn pathogen of major significance. Consumption of food contaminated with *Listeria* can cause both sporadic illness as well as foodborn disease epidemic (17,21,22). Listeriosis has been recognized as a rare but often fatal illness. In adults, the disease is characterized by the onset of severe symptoms including meningitis, septicemia, primary bacteremia, endocarditis, nonmeningitic central nervous system infection, and flu-like illnesses. People identified at high risk are pregnant women, neonates, organ-transplant recipients or those receiving immunosuppressive therapy (4,15,17).

The foodborn yeast and molds (fungi) includes several hundred species (1,3,23). The ability of these organisms to grow in many foods is due, in large part, to their relatively versatile environmental requirements. Both yeast and molds cause various degrees of deterioration and decomposition of food (3,17,18). In addition, some foodborn molds may be hazardous to human health because of their ability to produce toxic metabolites known as mycotoxins (3,23). Most mycotoxins are stable compounds that are not destroyed during food processing or home cooking. Even though the toxin generating organisms may not survive food preparation, the preformed toxin may still be present. Certain foodborn molds

may also elicit allergic reactions or may cause infection (1). In contrast to moulds, yeast is not harmful for human health.

Bacteriological contamination can occur from a wide variety of sources (5,24). Microorganisms are widely distributed in the products of animal origin. All foods possess a finite risk of microbiological contamination. The highest risk factors include foods of animal origin and foods consumed without prior cooking (5,25). Lori cheese is one of those kinds of products. At the same time, pathogenic organisms may also be transferred to food by food handlers either directly or by cross-contamination (24,26). In the chain of food distribution (26) (Figure1) food handlers who do not adequately wash their hands or use the wrong techniques to store food items provide one of the most common mechanisms of microbiological contamination (27). Moreover, with the current technology of food production and distribution, large numbers of people are exposed to the causative agent if a link in the food chain is contaminated (26,28,29). Thus, identifying, minimizing or eliminating the risk associated with the production, processing, transporting, storing and delivery of food products is an important matter for public health concerns (26,29).

These reasons dictate careful assessment of Lori cheese pathogenic contamination in the open-air markets of Yerevan. The purpose of this study was not to reveal the point of contamination in the chain of production and distribution of Lori cheese, but, as a first step, to assess the microbial safety of that product, sold in the open-air markets of Yerevan.

This study answered the research question: Does Lori cheese, sold in selected stores of Yerevan, conform to US and Armenia food safety standards (Table 1)?

The following are the major sources of microbial contamination of cheese, which appear to be a public health concern: coliforms, members of Enterobacteriaceaecea family, *Escherichia coli*O157:H7, *Listeria monocytogenes*, and molds that produce toxins (1,22,30). Contamination with salmonella is more common for eggs and poultry (3). However,

considering the possible cross contamination in open-air markets, due to their sanitary conditions, Lori cheese could be additionally contaminated with salmonella species. Thus salmonella became the subject of this study interest as well. Taking into account all mentioned above, the objectives of the study were:

1. Provide current data on the prevalence and levels of total coliforms, Enterobacteriaceae, *Escherichia coli*O157:H7, salmonella, *Listeria monocytogenes*, and molds in each of 60 samples of Lori cheese sold in Yerevan open-air markets during September 2003.
2. Publicize the information from microbiological analysis to the public about significance of health and environmental risk in order to provide further actions toward its minimizing.

2. METHODS

The project was a preliminary investigation of baseline bacteriologic data for the assessment of food safety, particularly Lori cheese. The program determined if the results of microbiological analysis are in acceptable ranges established by USDA (Table 2). This was a comprehensive survey with cross-sectional analytical study design, which could be used as a baseline for future inspection efforts. Open-air markets as the subject for the study, were selected because it is a place from which majority of populations prefer to consume a food.

Sample size of 60, for this study, was determined based on the following assumptions: (a) previous studies data about coliform and Enterobacteriaceae cheese contamination of 80% in cheese manufactures, (b) true proportion estimation within 10% (a range of 20%, between 70% to 90%), and (c) that the study results will be statistically significant within 95% confidence intervals.

Samples of Lori cheese were purchased only from open-air markets and included only products sold without packaging. Such criteria have been chosen in order to fairly assess the environmental contamination of a specified food traded in Yerevan markets, because absence

of refrigeration and unsanitary condition for food storage in public food markets contributes the growth of pathogenic microorganisms and their toxins (12). Moreover, unpacked product is a target for additional contamination since large number of people selects products by touching.

2.1. Sampling Methods

Sixty open-air markets of 12 Yerevan districts (5 from each district) were visited during a two-week period for microbial data collection. Once a district or neighborhood had been selected, 5 samples were collected.

A record was made for all samples of the times of day, date, consecutive numbers and locations of collections made. A sample unit consisted of a minimum of 150g that had been put in a plastic package at the market by the vendor. Sterile packages were not used in order to duplicate the actual condition of products purchased by consumers. Samples were taken at random to ensure that a sample is representative of the lot. To minimize the possibility of additional contamination, each purchased sample was analyzed the same day. Five samples were collected and analyzed every day. Sample analyses were performed for 12 days according to the schedule summarized in Table 2. Samples were analyzed for six indicators: coliforms, Enterobacteriaceae, *Escherichia coli* O157:H7, salmonella, *Listeria monocytogenes,* and molds.

Table 2. Analysis Schedule

Sampling date	Enrichment	Plating	Pick colonies	Inoculate biochemical	Read/ perform test
Monday	Monday	Tuesday	Wednesday	Thursday	Friday
Tuesday	Tuesday	Wednesday	Thursday	Friday	Monday
Wednesday	Wednesday	Thursday	Friday	Monday	Wednesday
Thursday	Thursday	Friday	Monday	Tuesday	Thursday
Friday	Friday	Saturday	Monday	Tuesday	Thursday

2.2. Analytical Methods

All sample units were examined for all six indicators, even though the food might have undergone heat or salt treatment and the microorganisms involved might no longer been viable. Completion of analyses was not dependent on the sample's organoleptic condition (appearance, smell, and feel).

Aseptic techniques were used in all analyses (after collection) in order to not introduce contamination during the handling and analytic procedures. Sterile instruments were used for cutting, removing and manipulating all samples. Samples were weighed in sterile containers that were used for dilution, and mixing (31,32).

Twenty-five gram sub-samples (the analytical unit) were used for Salmonella and *Listeria monocytogenes*, coliforms, Enterobacteriaceae, *Escherichia coli* O157:H7, and molds detection. All analytical units were taken from both outer and interior surfaces of each 150g sample unit to form a clear picture of food quality. Homogenous 1:10 dilution with sodium citrate and consecutive dilutions up to 1:10,000 with sterile 0.9% NaCl were made for inoculation and counting of pathogens in each specific media for, coliforms, Enterobacteriaceae, *Escherichia coli*O157:H7, and molds.

Classical microbiological techniques for pathogens, and molds detection for all analyses of 60 samples were performed (31) (Figure 2). Confirmation of results was done by biochemical testing for all pathogens and by simplified method of analysis using 3M's Petrifilm ™ plates for coliforms and Enterobacteriaceae (31) (Figure 2). For all pathogens a low serial dilution was used to prepare slides for direct microscopic examination.

Enumeration of Total Coliforms and Members of Enterobacteriaceae Family

Coliforms and Enterobacteriaceae enumeration was done by traditional microbiological methods and by 3M Petrifilm plate performance (20). In the assay, serial dilutions of samples were inoculated into Kessler and Brilliant Green Lactose Bile (BGLB) broth, 2%,

and incubated for 48 hours at 37°C for coliforms and Enterobacteriaceae enumeration. Control tube (without inoculation) for each broth was also incubated to compare the results. Coliforms, as well as members of Enterobacteriaceae family have specific ability to ferment lactose. Turbidity (changes in color and transparency compared with control) of broth showed presence of fermentation. A gas positive tube was considered as containing coliforms and Enterobacteriaceae. The numbers of contaminated tubes as well as the number of colonies on inoculated Petrifilm plates were counted. Inoculation in two types of enrichment broth media was done for precise calculation of data. Inoculation of 1ml 1:1,000 for Enterobacteriaceae and 1:10,000 dilutions for coliforms on Petrifilm plates helped in precise counting and registration of colonies.

Total coliforms and Enterobacteriaceae colony counting technique on Petrifilm plates

Counting and registration of colonies were done by the two following techniques (12):

1. When the number of colonies did not exceed 200, the whole surface of Petrifilm plate was counted.
2. When the number of colonies was estimated to be larger than 200, the Petrifilm plate grid was used, by counting the number of colonies in one grid and multiplying it by 20 (the total number of Petrifilm plate grids).

The number of colonies per plate for each sample of cheese was registered (Appendix 2).

Total coliforms and Enterobacteriaceae colony calculating technique in sample unit (cfu/g)

Both, for total coliforms and members of Enterobacteriaceae family, when the total number of colonies in sample unit exceeded 10^7 cfu/g, the exact quantity of colonies was not counted. The level of contamination 10^7 cfu/g for sample was estimated when 50 colonies per grid for coliforms and 500 colonies per grid for Enterobacteriaceae were counted. Those samples was considered as very high in contamination and were registered as > 1000 colonies in 1:10,000 dilution for coliforms and > 10,000 colonies in 1:1000 dilution for members of

Enterobacteriaceae family. For all other samples, the exact number of colonies per sample were registered (Appendix 2).

For total coliforms count, according to the US critical control point of cheese safety, only samples, where on Petrifilm plates no colony growth was registered, were considered safe. Otherwise, even if one colony was registered,, the sample was considered as unsafe.

For Enterobacteriaceae count, taking into consideration the dilution level of sample units and assumption that in 1:10,000 dilution absence of Enterobacteriaceae family members is safe, the samples where colonies count did not exceed 9 were considered as safe.

Detection of *Escherichia coli*

To identify *E. coli*, transference from each 1:10,000 diluted gassing tube of Kessler and Brilliant Green Lactose Bile broth by loop to a specific for *E. coli* growth media (Endo) plate, was done. All plates were examined after 18-24 hours incubation at 44°C for suspicious *E. coli* colonies, i.e., dark centered and flat, with or without metallic sheen. Transference of at least 2 suspicious colonies from each Endo plate was used for biochemical testing of all *E. coli* which include IMViC reactions (combination of four biochemical tests that are described bellow), ß-galactosidase, and oxidase tests and Gram stain performance as Gram-negative cultures with short rods. Transference of 5 colonies for specific *E. coli* O157:H7 onto MacConkey Sorbitol Agar (MSA) and further oxidase and sorbitol biochemical confirmation tests were used in order to confirmthe existence of this specific strain.

IMViC reactions performance

Indole production. Tube of tryptone broth was inoculated and incubated 24 ± 2 hours at 35°C. Testing for indole by adding 0.2-0.3 mL of Kovacs' reagent was performed. Appearance of distinct red color in upper layer verifies positive test (+).

Voges-Proskauer (VP)-reactive compounds. Tube of MR-VP broth was inoculated and incubated 48 ± 2 hours at 35°C. One mL was transferred to 13 x 100 mm tube; added to it was 0.6 mL α-naphthol

solution, 0.2 mL 40% KOH, and a few crystals of creatine, which was then left to stand for 2 hours. Test was positive (+) if eosin pink color developed.

Methyl red-reactive (MR) compounds. After additional 48 ± 2 hours at 35°C incubation of MR-VP tube for VP test 5 drops of methyl red solution to each tube were added. Distinct red color was positive test (+). Yellow suggests negative reaction (-).

Citrate. Lightly inoculated tube of Koser's citrate broth was incubated for 96 hours at 35°C. Development of distinct turbidity presented positive reaction (+).

In order to be considered as contaminated with *E. coli*, a sample was observed with cultures that (a) appear as Gram-negative nonsporeforming rods, (b) ß-galactosidase positive(+), oxidase negative(-), and (c) give patterns of IMViC for VP positive (+), MR negative (-), and Citrate negative (-) (17,20).

The strains of *E. coli* were considered as *E. coli* O157:H7 when uncolored yellow colonies, negative on oxidase test and negative on sorbitol, grew on McConkey Sorbitol Agar.

Detection of Salmonella

Analyses of 25-g analytical unit at random from each 150-g sample unit for the presence of salmonella using Buffered Peptone broth, Rappaport-Vassiliadis (RV) medium, Bismuth sulfite (BS) Agar, and Improved Salmonella Agar were performed. After 24 ± 2 hours at 35°C incubation, plates had been examined for presence of colonies that may be salmonella (18). Brown, gray, or black colonies, sometimes with a metallic sheen, area sign of salmonella. In addition, specific reactions to identify salmonella by fermentation of lactose, glucose, and sucrose, as well as urease-, indol-, VP-, H_2S-, and b-galactosidase tests were performed according to international standards of microbiological testing (17,18,21). These 10 consequent tests were doneto confirm the results of microbial analyses and to be sure in validity and reliability of salmonella testing results.

The following patterns in table 3 (18) helped to classify cultures as salmonella.

Table 3. Biochemical reactions of salmonella

Test or substrate	Result		Salmonella species reaction([a])
	Positive	Negative	
1. Glucose (TSI)	yellow butt	red butt	+
2. b-galactosidase test	yellow butt	no color change	+
3. H_2S (TSI and LIA)	blackening	no blackening	+
4. Urease	purple-red color	no color change	-
5. Indole test	violet color at surface	yellow color at surface	-
6. Phenol red lactose broth	yellow color and/or gas	no gas; no color change	-
7. Phenol red sucrose broth	yellow color and/or gas	no gas; no color change	-
8. Voges-Proskauer test	pink-to-red color	no color change	-

[a] +, 90% or more positive in 1 or 2 days; -, 90% or more negative in 1 or 2 days.

Although in some tests, for example, glucose fermentation, Voges-Proskauer, and H_2S, a marked number of samples appeared as suspicious for salmonella presence, only the typical salmonella appearance combination of positive and negative outcomes for all tests was considered as a final rule for decision making.

Detection of *Listeria monocytogenes*

Analytical portion (25 g) was tested with pre-enriched for *Listeria* species at 37° C for 4 hours in Frizer Broth Base than streaked onto esculin-containing selective isolation agar PALKAM and incubated at 37°C for 48 hours. (22). Listeria colonies are black with a black halo on esculin-containing media (32). Typical colony from culture plate was examined using microscope. They also were used for conventional fermentation method in which Listeria has ability to rhamnose, xylose fermentation, and no ability in manit fermentation (32). Nitrate (negative for Listeria monocytogenes) and lycitinase (positive for Listeria monocytogenes) activity tests were also performed to confirm the results. Gram stain performance for 16- to 24-hour cultures was done (all Listeria species are gram-positive).

Detection of Molds and Yeast

Molds and yeast were detected by simple inoculation of 0.1ml of 1:10 dilution of analytical unit onto specific Chapeck medium and incubated at 25°C for 5 days (12). If there was no growth after 5 days of incubation, plates were incubated for another 48 hours to allow heat- or chemically stressed cells and spores enough time to grow. Toxic and allergic molds were specified and number of contaminated plates counted.

2.3. Ethical Consideration

The research proposal was reviewed and approved by the Student Project Institutional Review Board (IRB) within the American University of Armenia's College of Health Sciences. There were no human subjects involved, as this is a study related to product testing.

3. RESULTS

Cheese samples were collected and analyzed for the presence of microorganisms and were considered as safe or unsafe for consumption according to USDA critical limits of food safety standards for specific pathogens (Table 1).

3.1. Data for Coliforms

In this study, from 60 analyzed samples of Lori cheese, in 85% (51/60) of samples the total coliforms counts dramatically exceeded the USDA and Armenian standard for safety(Table 4, Appendix 2). In those 51 samples, the total coliform counts were between 1 and >1,000 cfu[1]/g in 1:10,000 dilution and were distributed as showed in Table 4.

[1]Colony forming unit (the # of bacteria that form colonies in growth media. The total # of original bacteria will be larger.)

Table 4. Number of Coliforms Counted on Petrifilm in 1:10,000 dilution

# of coliforms (cfu/g)	Frequency	Percent
>1,000	9	15
≥100 <999	9	15
≥10 ≤99	18	30
≥1 ≤9	15	25
Total unsafe	**51**	**85**
0	9	15
Total safe	**9**	**15**
Total	60	100

Among 51 unsafe samples, 71% were considered heavily contaminated with coliforms (e.g. number of colony forming units exceeded 10×10^4), which means if a sample is contaminated it is more likely to be heavily contaminated. In 15 samples (29%), from 1 to 9 developed colonies were registered, which is also unsafe. And only in 9 samples (15%) did the coliform count not exceed the limit and were identified as safe according to USDA critical standards (Table 4) (Figure 3). The results correlated for all methods used: if no changes were discovered in Kessler and BGLB broth enrichment media, no pathogen growth was registered in Petrifilms as well.

The results showed a broad geographic distribution of contaminated samples across all 12 districts of Yerevan (Figure 4). Although some samples from some districts had been contaminated less than others, no geographic clustering or free of contamination areas were detected (Figure 4, Appendix2).

3.2. Data for Members of Enterobacteriaceae Family

Although specific USDA guidelines in terms of members of the Enterobacteriaceaeceae family have not yet been set, the counts of these microbiota were also very high: 70% (42/60) (Table 5) (Figure 3), considering absence in 1:10,000 dilution as safe. According to this assumption of safety, the distribution of safe and unsafe samples was the following:

Table 5. Number of Enterobacteriaceae Counted on Petrifilm in 1:1,000 Dilution

# of Enterobacteriaceae (cfu/g)	Frequency	Percent
>10,000	8	14
≥1,000 <9,999	5	8
≥100 ≤999	12	20
≥10 ≤99	17	28
Total unsafe	**42**	**70**
≥0 ≤9	18	30
Total safe	**18**	**30**
Total	60	100

Among 42 samples considered unsafe for Enterobacteriaceae, 60% were heavily contaminated with Enterobacteriaceae (number of colony forming units exceeded 100). In 17 samples (40%), from 10 to 99 developed colonies were registered and also were considered as unsafe. In 18 samples (30%), Enterobacteriaceae counts did not exceed the safety threshhold (Table 4) (Figure 3). In 13 samples, the number of cultivated members of Enterobacteriaceae family in 1:1,000 dilution on Petrifilms, was less than the number of cultures in 1:10,000 dilution for coliforms.

Geographical distribution of contaminated samples was similar to that of coliforms.

3.3. Data for *E. coli* O157:H7

In this study high prevalence of *Escherichia coli* was detected. Four samples out of 60 were contaminated with E. coli (6.7%) (Table 6).

Table 6. Samples Tested Positive with *E. coli* and *E. coli* O157:H7 strain

Location	# of Coliform colonies in 1ml 1:10,000 dilution	# of Enterobacteriaceae colonies in 1ml 1:1,000 dilution	*E. coli* presence	*E. coli* O157:H7 presence
Davitashen	23	80	Detected	Not Detected
Kentron	700	90	Detected	Not Detected
Kentron	>1,000	>10,000	Detected	Detected
Nubarashen	6	52	Detected	Detected

Among those 4 samples with *E. coli*, 2 were detected as *E. coli* O157:H7 strain. The contaminated samples were collected from Kentron and Nubarashen districts and besides being contaminated with *E. coli* were heavily infected with coliforms and members of the Enterobacteriaceae family as well (Table 6).

3.4. Data for Salmonella

All analytical samples complied with the salmonella performance for safety (absent of salmonella in 25g analytical unit). No Salmonella was registered in any of 60 samples of analysis, even though, there were some samples that appeared positive for salmonella in one or two biochemical reactions (Appendix 2).

3.5. Data for *Listeria monocytogenes*

Listeria monocytogenes was detected in two samples (3.3%). As it was done for salmonella, the series of biochemical testing were performed to clearly identify the *Listeria monocytogenes* in analyzed portion of the sample. The contaminated samples were collected from Avan and Nork-Marash districts (Figure 4, Appendix 2).

During *Listeria monocytogenes* identification two samples with other types of health threatening pathogens, not part of the study protocol, were discovered. The exact identification of those strains was not been performed due to the goal of this particular study and some technical complications.

3.6. Data for Molds

Specific USDA guidelines in terms of molds have not yet been set. However, the counts of these substances can also be considered as high: 20% (Table 7). The most toxic molds, in the mold contaminated samples were *Aspergillus fumigatus* and *Aspergillus ochraceus*. They produce tremorgenic toxin and ochratoxin respectively and were found in 4 samples. *Penicillium*, *Cladosporium herbarum*, and *Niger*, molds that could cause allergies, were found in 8 samples (Appendix 2). Yeast was detected in all samples of analysis.

Distribution of different types of mold in analyzed samples presented bellow (Table 7); no samples contained both toxic and allergenic molds.

Table 7. Distribution of mold types among samples

Types of molds	Frequency	Percent
Only Toxic	4	6.7
Only Allergenic	8	13.3
Total contaminated samples	**12**	**20**
Total	60	100

4. INTERPRETATION

The result of bacteriological analyses showed heavy contamination of samples with coliform bacteria and members of Enterobacteriaceae family (Figure 3). To be confident in obtained results multiple techniques of pathogen detection were used. The presence of such pathogens show the poor sanitary condition of Lori cheese sold in open-air markets of Yerevan and possible presence of other type of pathogens.

Presence of *E. coli* in 6.7% of analyzed samples mean that the problem of sample contamination with *E. coli* was significant. The severity of health problems caused by particular strain of *E. coli* O157:H7 makes the presence of microorganism in consumed food amatter of serious concern.

High counts per gram of cheese for coliforms and members of the Enterobacteriaceaeceae family and presence of *E. coli* suggest that the Lori cheese sold in Yerevan open-air markets, most probably, have fecal contamination. Improper milking hygiene without subsequent pasteurization of milk and the lack of general food–hygiene-related knowledge and infrastructure of marketing could be the sources and causes of such contamination. This also indicates that a large fraction of the Lori cheeses probably contain unsafe levels of total coliforms and *E. coli* at the time of consumption.

Salmonella was not detected in any sample, even though the series of tests were performed in order to be confident of reaction results. Salmonella species are not very typical and not common for cheese (3).

In this study, microbial testing identified presence of *Listeria monocytogenes* in 2 samples. According to *Listeria monocytogenes* identification test results, some assumption for presence of other types of health-threatening pathogens in examined samples could be made. Arrival of 4 negative (-) reactions for manit test and 3 negative (-) reactions for nitrate reduction test, showed the existence of other than *Listeria monocytogenes* strains of Listeria in analytical samples. Those strains also could be causative agents for disease development, and their presence could be considered as an indicator of an unsafe sample. Appearance of 27 negative results (-) for xylose test might be a reason for presence of other gram-stain pathogens such as *Staphylococcus* or other coccoidal forms of microorganisms that do not ferment the xylose as *Listeria* do. However, the identification of specific strains of microorganisms always assumed comparative use of known culture and modern and more selective methods of analyses (33,34) and required more time and money than were available.

Lori cheese is a type of soft cheeses where the presence of any kind of yeast and molds is unusual. Thus the 20% contamination of samples with toxic- and allergy-causing molds should raise concern (Table 7) and can be viewed as an indicator of unsafe environmental conditions during cheese production and distribution.

5. CONCLUSION

This study showed presence of all type of examined pathogens except salmonella in analyzed samples of Lori cheese sold in open-air markets of Yerevan (Table 8).

Table 8. Microbial distribution in 60 analyzed samples above the US and Armenia critical limits for safety

Pathogens	Frequency	Percent
Coliforms	51	85
Enterobacteriaceae	42	70
Generic *E. coli*	4	6.7
E. coli O157:H7	2	3.3
Salmonella	0	0
Listeria monocytogenes	2	3.3
Molds (toxic & allergic)	12	20
Other health-threatening pathogens	2	3.3

Due to some limitations of this study, such as absence of comparison group from a Lori cheese manufacturing site, it is difficult to determine the source(s) of contamination in the chain of Lori cheese production and distribution (Figure 1). Although, information about Lori cheese producers was collected (Appendix 2), it sill unclear if the cheese was produced by manufacturers or by local farmers. Taking into consideration the lack of sanitary condition in farming, milking, and product distribution processes it might be useful to examine samples of Lori cheese on staphylococcal contamination, but the lack of time and resources did not allow such analyses. According to the results of this study, people consume unsafe food that may lead to disease.

The findings of this study emphasize the elevated microbial counts resulting from insufficient training about proper farming, milking, handling, and selling practices lead to cheese as a sure medium of food-related infection. In spite of the fact, that only few samples were identified as contaminated with *E. coli* O157:H7 and *Listeria monocytogenes*, the magnitude of the health problems caused by these microorganisms make the findings critical and becomes the basis for recommendations and prompt action to implement improved sanitary conditions and monitoring of food production, distribution, storage, handling, and sales..

Results of laboratory tests on Lori cheese disclosed unsatisfied sanitary conditions of products sold in open-air markets of Yerevan and are adequate grounds for inspection and other appropriate measures to reduce the prevalence of pathogens.

There is a need to improve inspection infrastructure to carry out its regulatory responsibilities more effectively and efficiently and to be in a position to deal with products having animal origin. It is necessary to follow the written governmental food safety program and to develop appropriate sanitation programs for the market level in order to allow the monitoring and recall of unsafe food as appropriate.

It is very important to place more emphasis on professional education, which will help to bridge the gap between what inspection personnel had to know under the previous system and what they have to know now. Moreover, it is necessary to ensure that all food handlers have skills and competencies in food hygiene matters coequal with work activities. A better educated consumer will also drive demands for safe, quality food.

Sanitary organizations must monitor the whole process of production and distribution of cheeses to ensure it safety and to report the results monthly to the consumers. Inspectors from central sanitary organizations should work with local officials to determine the best way to work together in order to provide public with safe food in all districts. It is important to make sure that the food safety gained, if any, within plants are not lost due to mishandling during distribution.

Markets should be required to ensure they are meeting their responsibility to keep their facilities and equipment clean and safe. In addition, they should conduct microbial testing for generic *E. coli* to verify that their control systems are working to prevent fecal contamination, a primary avenue of contamination of raw product with harmful bacteria (33).

For further studies, it is important to consider larger sample size and more time for expanded analyses. To fairly assess the contamination due to marketing, it will be more

appropriate to have comparison group in the manufacturing level of food distribution chain. Moreover, it could be interesting to compare the level of sample contamination at different time of year in order to clarify influence of weather on the level of contamination.

And at last, it could be useful to examine analytical samples on existence of staphylococcal infection as common pathogen for milk and milk products because the toxins produced by those organisms are very harmful to human health. It is also important to do analyses of physical parameters of Lori cheese, such as pH, water activity, and the level of NaCl to find correlation between those and the level of microbial contamination.

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Figure 1. Distribution model

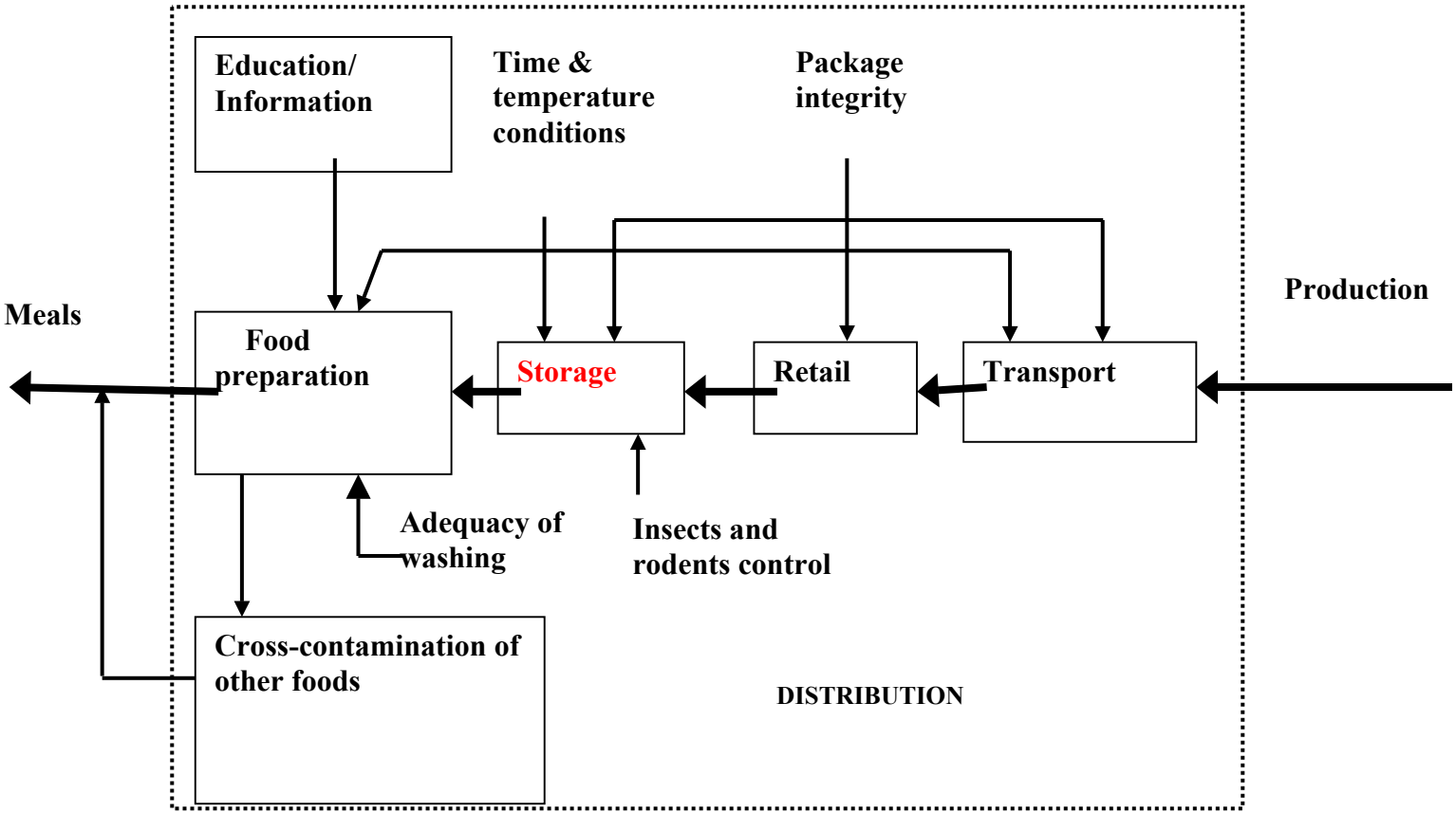


Figure 2. Systematic scheme for microbial testing of Lori cheese samples using conventional methods

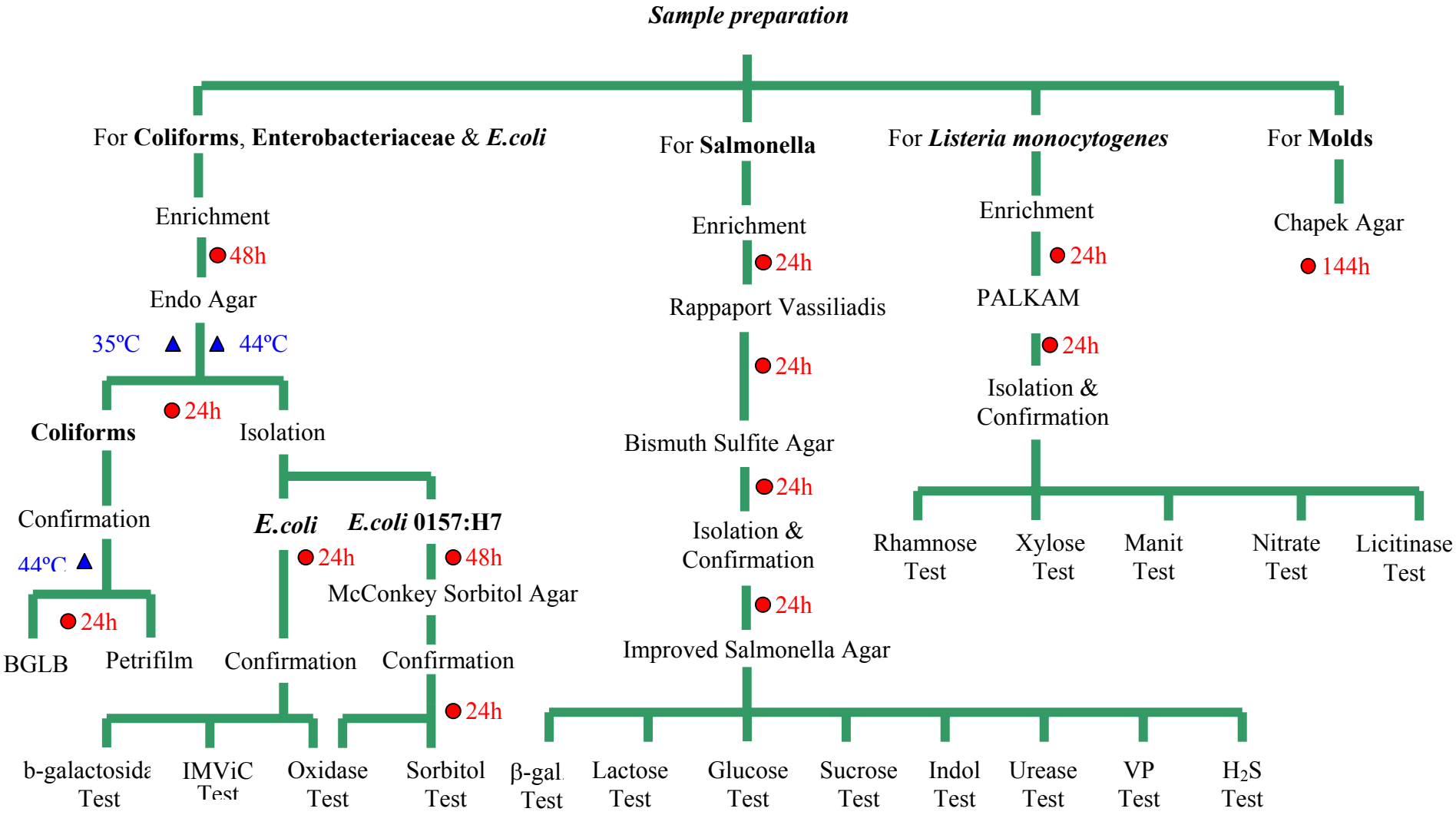
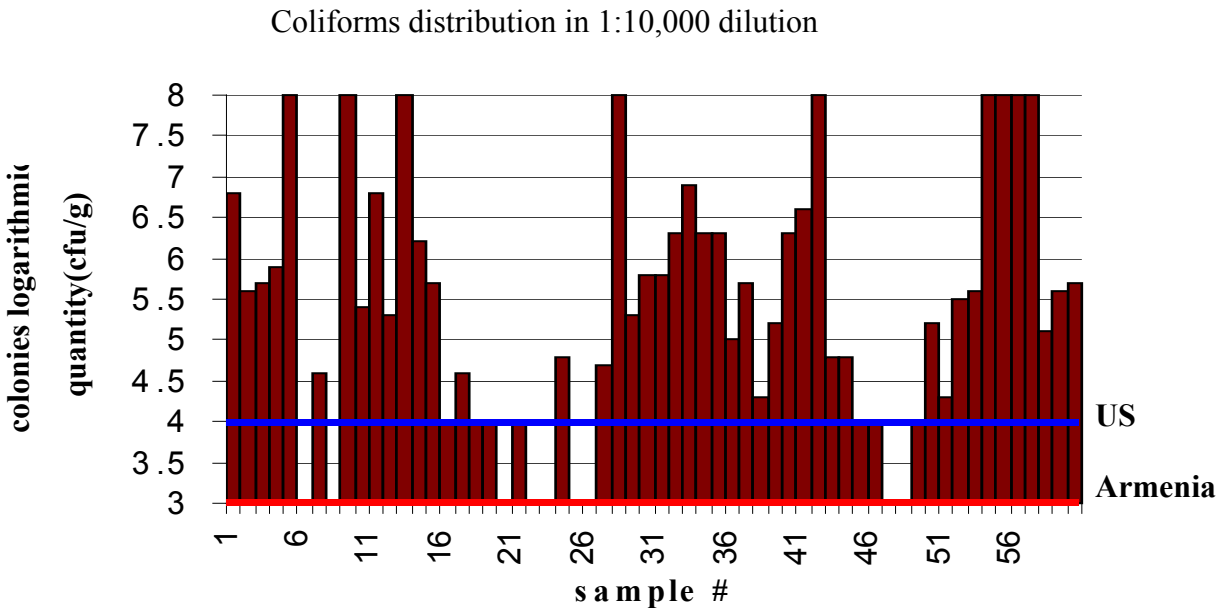


Figure 3. The graphical mapping of coliforms and Enterobacteriaceaecea distribution in 60 samples of Lori cheese



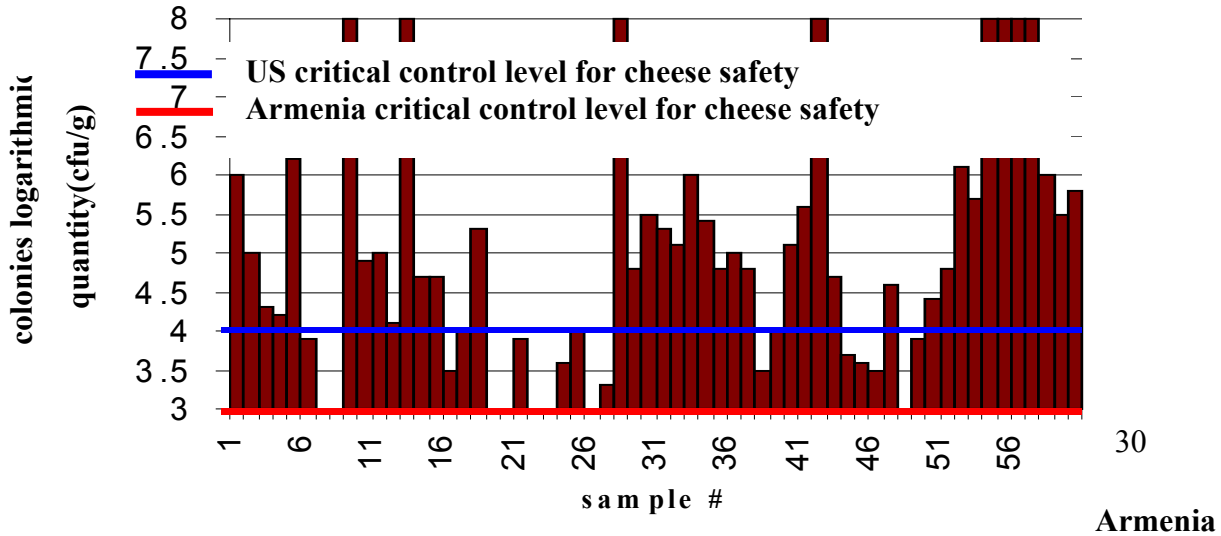
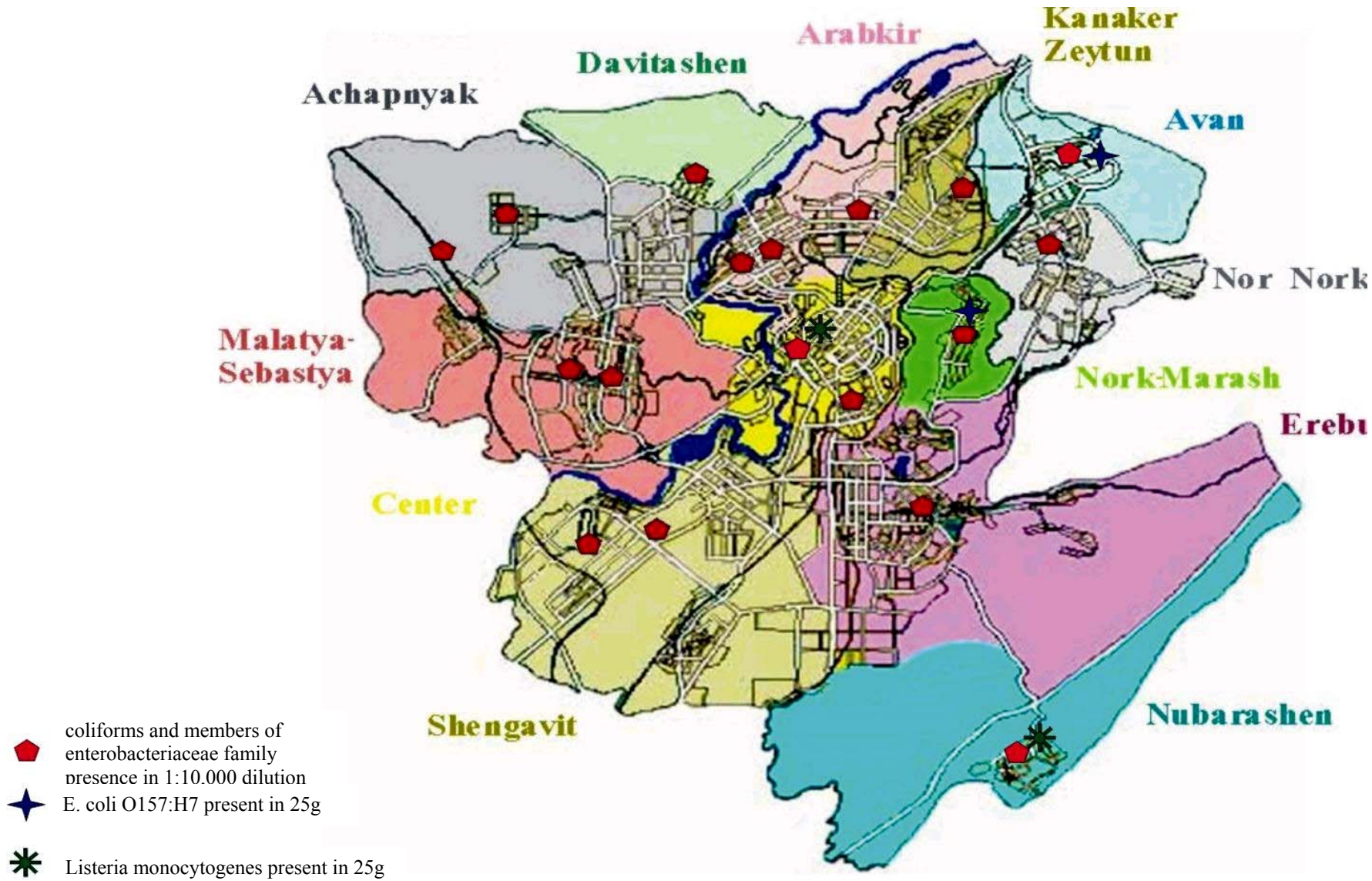


Figure 4. Distribution of contaminated samples of Lori cheese in districts of Yerevan



APPENDIX 1. Characteristics of analyzed pathogens

Salmonella

Disease, Symptoms and Onset	Causes acute diarrhea, vomiting, abdominal pain, and fever. Occasionally, may cause blood stream infections and death. Symptoms occur 6 to 72 hours after eating contaminated food.
Main Disease Factor	Invasion of the lining of the intestine.
Source	Fecal contamination of meat and poultry.
Transmission	Primarily from consumption of raw or undercooked eggs, milk, meat and poultry.
Characteristics	• Killed by mild heat. • Grows with or without air. Grows best at human body temperature. Grows very poorly at refrigeration temperatures and does not grow above 130°F. • Does not grow well or at all in acidic foods. • Survives well in frozen or dry foods. Bacteria in dry foods are more resistant to heat.

***Escherichia coli* O157 :H7**

Disease, Symptoms and Onset	Causes diarrhea (may be bloody) and occasionally fever. Incubation period is generally 2-3 days after ingestion of food (range 1-5 days). May result in kidney failure and death, especially in children.
Main Disease Factor	Production of a potent toxin in the intestinal tract of infected people.
Source	Fecal contamination of beef and poultry.
Transmission	Consumption of raw or undercooked meat or poultry, contaminated produce, such as sprouts, unpasteurized milk, and juices.
Characteristics	• Killed by mild heat. • Grows with or without air. Optimum temperature for growth is human body temperature. • Grows in moist, low-acid foods.

Listeria monocytogenes

Disease, Symptoms and Onset	Causes meningitis (sudden fever, intense headache, nausea, vomiting, delirium and coma). This is a particular problem in the elderly, infants, and pregnant women. One third of those who are hospitalized will die. In a healthy person, infection with *Listeria monocytogenes* may cause symptoms such as a flu-like illness and diarrhea.
Main Disease Factor	Bacterial invasion of the blood stream.
Source	Post-heat-processing contamination from the plant environment including plant personnel, equipment, floors, walls, drains, condensation from coolers, etc.
Transmission	Consumption of contaminated processed ready-to-eat meats. Also vegetables, unpasteurized dairy products.
Characteristics	• Killed by pasteurization temperatures. • Grows with or without air – however, prefers reduced oxygen conditions. • Able to grow at refrigeration temperatures and high salt concentration. • Acid conditions will slow growth but may allow survival. • Extremely hardy in comparison to most bacteria. Withstands repeated freezing and thawing. Survives for prolonged periods in dry conditions.

APPENDIX 2. The database of "BASELINE ASSESSMENT OF THE MICROBIAL CONTAMINATION OF LORI CHEESE SOLD IN YEREVAN MARKETS"

ID	Producer	Molds	Total coliforms in Kessler media broth		Total coliforms in BGLB media broth		Performance on Petrifilm in 1ml of 1:10000 dilution (>0 = contaminated)		Performance on Petrifilm in 1ml of 1:1000 dilution (>10 = contaminated)	
			Inoculation from **1:1000** delution	Inoculation from **1:10000** delution	Inoculation from **1:1000** delution	Inoculation from **1:10000** delution	**# of coliform colonies**	**Samples safety for coliforms**	**# of Enterobacter.colonies**	**Samples safety for Enterobact.**
31	Kalinino	yeast	+	+	+	+	650	unsafe	1000	unsafe
32	Vardenis	yeast	+	+	+	+	47	unsafe	110	unsafe
33	Karmir	Aspergillus fumigatus	+	+	+	+	50	unsafe	20	unsafe
34	Karmir	yeast,Cladosporium herbarum	+	+	+	+	90	unsafe	14	unsafe
35	Sisian	yeast	+	+	+	+	>1000	unsafe	1500	unsafe
41	Kalinino	Aspergillus ochraceus	+	+	+	+	0	safe	8	safe
42	Dumikyan	yeast	+	+	+	+	4	unsafe	0	safe
43	Kalinino	yeast,Penicillium	-	-	-	-	0	safe	0	safe
44	Kalinino	yeast,Cladosporium herbarum	+	+	+	+	>1000	unsafe	>10000	unsafe
45	Kalinino	yeast,Cladosporium herbarum	+	+	+	+	23	unsafe	80	unsafe
11	Vardenis	yeast,Aspergillus fumigatus	+	+	+	+	700	unsafe	90	unsafe
12	Akhlkala	yeast	+	+	+	+	20	unsafe	13	unsafe
13	Kalinino	yeast,Niger	+	+	+	+	>1000	unsafe	>10000	unsafe
14	Sarnakun	yeast	+	+	+	+	150	unsafe	45	unsafe
15	Sisian	yeast	+	+	+	+	55	unsafe	45	unsafe

ID	Coliforms and E.coli growth on Endo selective media	Reactions for E.coli presence					E. coli O157:H7 growth on selective McConkey Sorbitol Agar Base
		Test on b-galactosidase reaction should be (+)	**Test IMVIC** result should be (+)	**Test on oxidase** reaction should be (-)	**Test on sorbitol** reaction should be (-)	**Gram stain performance** reaction should be (-)	
31	+	-	-	-	+	-	-
32	+	-	-	+	+	-	-
33	+	-	-	-	+	-	-
34	+	-	-	-	+	-	-
35	+	-	-	-	+	-	-
41	+	+	-	-	+	-	-
42	+	-	-	-	+	-	-
43	+	-	-	-	+	-	-
44	+	+	+	-	+	-	-
45	+	+	+	-	+	-	+
11	+	+	+	-	+	-	+
12	+	-	-	-	+	-	-
13	+	+	+	-	-	-	+
14	+	+	-	-	+	-	-
15	+	+	-	-	+	-	-

ID	Salmonella growth on selective Bismut Sulfit Agar	Salmonella growth on selective Improved Salmonella Agar	Reactions for salmonella presence								
			Test on b-galactosidase reaction should be (+)	**Test on lactose** reaction should be (-)	**Test on glucose** reaction should be (+)	**Test on sucrose** reaction should be (-)	**Test on indol** reaction should be (-)	**Test on VP** reaction should be (-)	**Test on urease** reaction should be (-)	**Test on H2S** reaction should be (+)	**Gram stain performance** reaction should be (-)
31	-	-	-	+	-	+	+	+	+	-	-
32	-	-	-	+	-	+	+	+	+	-	-
33	-	-	-	+	-	+	+	+	+	-	-
34	-	-	-	+	-	+	+	+	+	-	-
35	-	-	-	+	-	+	+	+	+	-	-
41	-	-	-	+	-	+	+	+	+	-	-
42	-	-	-	+	-	+	+	+	+	-	-
43	-	-	-	+	-	+	+	+	+	-	-
44	+	-	-	+	+	+	+	-	+	+	-
45	-	-	-	+	-	+	+	+	+	-	-
11	+	-	-	+	+	+	+	-	+	+	-
12	-	-	-	+	-	+	+	+	+	-	-
13	+	-	-	+	+	+	+	-	+	+	-
14	+	-	-	+	+	+	+	-	+	+	-
15	-	-	-	+	-	+	+	+	+	-	-

ID	Listeria monocytogenes growth on selective media PALKAM	Reactions for Listeria monocytogenes presence					
		Test on rhamnose reaction should be (-)	**Test on xylose** reaction should be (-)	**Test on manit** reaction should be (-)	**Test on nitrate** reaction should be (-)	**Test on lycitinase activity** reaction should be (+)	**Gram stain performance** reaction should be (+)
31	-	+	+	+	+	-	-
32	+	+	-	+	+	-	+
33	-	+	+	+	+	-	-
34	-	+	+	+	+	-	-
35	+	+	-	+	+	-	+
41	-	+	+	+	+	-	-
42	-	+	+	+	+	-	-
43	-	+	+	+	+	-	-
44	-	+	+	+	+	-	-
45	-	+	+	+	+	-	-
11	+	+	+	+	+	-	+
12	+	+	-	+	+	-	+
13	-	+	+	+	+	-	-
14	+	+	-	+	+	-	+
15	-	+	+	+	+	-	-

ID	Producer	Molds	Total coliforms in Kessler media broth		Total coliforms in BGLB media broth		Performance on Petrifilm in 1ml of 1:10000 dilution (>0 = contaminated)		Performance on Petrifilm in 1ml of 1:1000 dilution (>10 = contaminated)	
			Inoculation from **1:1000** delution	Inoculation from **1:10000** delution	Inoculation from **1:1000** delution	Inoculation from **1:10000** delution	**# of coliform colonies**	**Samples safety for coliforms**	**# of Enterobacter.colonies**	**Samples safety for Enterobact.**
81	Amasia	yeast	+	+	+	+	1	unsafe	3	safe
82	Kalinino	yeast	+	+	+	+	4	unsafe	10	unsafe
83	Goris	yeast	+	+	+	+	1	unsafe	180	unsafe
84	Kalinino	yeast	+	+	+	+	1	unsafe	1	safe
85	Sisian	yeast	-	-	-	-	0	safe	0	safe
91	Vanadzor	yeast	+	+	+	+	1	unsafe	9	safe
92	Vanadzor	yeast,Mucor	-	-	-	-	0	safe	0	safe
93	Vardenis	yeast	+	-	+	-	0	safe	1	safe
94	Kalinino	yeast	+	+	+	+	6	unsafe	4	safe
95	Kalinino	yeast	+	+	+	+	0	safe	11	unsafe
51	Martuni	yeast	-	-	-	-	0	safe	0	safe
52	Talin	yeast	+	+	+	+	5	unsafe	2	safe
53	Kalinino	yeast	+	+	+	+	>1000	unsafe	>10000	unsafe
54	Kalinino	yeast	+	+	+	+	20	unsafe	65	unsafe
55	Hrazdan	yeast	+	+	+	+	70	unsafe	300	unsafe

ID	Coliforms and E.coli growth on Endo selective media	Reactions for E.coli presence					E. coli O157:H7 growth on selective McConkey Sorbitol Agar Base
		Test on b-galactosidase reaction should be (+)	**Test IMVIC** result should be (+)	**Test on oxidase** reaction should be (-)	**Test on sorbitol** reaction should be (-)	**Gram stain performance** reaction should be (-)	
81	+	-	-	-	+	-	-
82	+	-	-	-	+	-	-
83	+	-	-	-	+	-	-
84	+	+	-	-	+	-	-
85	+	-	-	-	+	-	-
91	+	+	-	-	+	-	-
92	+	+	-	-	+	-	-
93	+	+	-	-	+	-	-
94	+	+	-	-	+	-	-
95	+	-	-	-	+	-	-
51	+	-	-	-	+	-	-
52	+	+	-	-	+	-	-
53	+	+	-	-	+	-	-
54	+	-	-	-	+	-	-
55	+	+	-	-	+	-	-

ID	Salmonella growth on selective Bismut Sulfit Agar	Salmonella growth on selective Improved Salmonella Agar	Reactions for salmonella presence								
			Test on b-galactosidase reaction should be (+)	**Test on lactose** reaction should be (-)	**Test on glucose** reaction should be (+)	**Test on sucrose** reaction should be (-)	**Test on indol** reaction should be (-)	**Test on VP** reaction should be (-)	**Test on urease** reaction should be (-)	**Test on H2S** reaction should be (+)	**Gram stain performance** reaction should be (-)
81	-	-	-	+	-	+	+	+	+	-	-
82	-	-	-	+	-	+	+	+	+	-	-
83	+	-	-	+	+	+	+	-	+	+	-
84	-	-	-	+	-	+	+	+	+	-	-
85	-	-	-	+	-	+	+	+	+	-	-
91	+	-	-	+	+	+	+	-	+	+	-
92	+	-	-	+	+	+	+	-	+	+	-
93	+	-	-	+	+	+	+	-	+	+	-
94	+	-	-	+	+	+	+	-	+	+	-
95	+	-	-	+	+	+	+	-	+	+	-
51	+	-	-	+	+	+	+	-	+	+	-
52	+	-	-	+	+	+	+	-	+	+	-
53	+	-	-	+	+	+	+	-	+	+	-
54	+	-	-	+	+	+	+	-	+	+	-
55	+	-	-	+	+	+	+	-	+	+	-

ID	Listeria monocytogenes growth on selective media PALKAM	Reactions for Listeria monocytogenes presence					
		Test on rhamnose reaction should be (-)	**Test on xylose** reaction should be (-)	**Test on manit** reaction should be (-)	**Test on nitrate** reaction should be (-)	**Test on lycitinase activity** reaction should be (+)	**Gram stain performance** reaction should be (+)
81	-	+	+	+	+	-	-
82	+	+	-	+	+	-	+
83	+	+	+	+	+	-	+
84	+	+	-	+	+	-	+
85	-	+	+	+	+	-	-
91	-	+	+	+	+	-	-
92	-	+	+	+	+	-	-
93	+	+	-	+	+	-	+
94	+	+	-	+	+	-	+
95	-	+	+	+	+	-	-
51	-	+	+	+	+	-	-
52	+	+	-	+	+	-	+
53	+	+	-	+	+	-	+
54	-	+	+	+	+	-	-
55	-	+	+	+	+	-	-

ID	Producer	Molds	Total coliforms in Kessler media broth		Total coliforms in BGLB media broth		Performance on Petrifilm in 1ml of 1:10000 dilution (>0 = contaminated)		Performance on Petrifilm in 1ml of 1:1000 dilution (>10 = contaminated)	
			Inoculation from **1:1000** delution	Inoculation from **1:10000** delution	Inoculation from **1:1000** delution	Inoculation from **1:10000** delution	**# of coliform colonies**	**Samples safety for coliforms**	**# of Enterobacter.colonies**	**Samples safety for Enterobact.**
21	Azatan	yeast	+	+	+	+	63	unsafe	200	unsafe
22	Vardenis	yeast	+	+	+	+	200	unsafe	140	unsafe
23	Bogdanov	yeast	+	+	+	+	800	unsafe	1000	unsafe
24	Kalinino	yeast	+	+	+	+	220	unsafe	230	unsafe
25	Vardenis	yeast	+	+	+	+	190	unsafe	70	unsafe
101	Kalinino	yeast	+	+	+	+	10	unsafe	100	unsafe
102	Vanadzor	yeast	+	+	+	+	50	unsafe	70	unsafe
103	Sisian	yeast	+	-	+	-	2	unsafe	3	safe
104	Sisian	yeast,Niger	+	+	+	+	15	unsafe	11	unsafe
105	Kalinino	yeast	+	+	+	+	200	unsafe	120	unsafe
71	Kalinino	yeast,Asperdillus ochraceus	+	+	+	+	370	unsafe	400	unsafe
72	Sisian	yeast	+	+	+	+	>1000	unsafe	>10000	unsafe
73	Sisian	yeast	+	+	+	+	6	unsafe	52	unsafe
74	Goris	yeast	+	+	+	+	6	unsafe	5	safe
75	Kalinino	yeast	-	-	+	+	1	unsafe	4	safe

ID	Coliforms and E.coli growth on Endo selective media	Reactions for E.coli presence					E. coli O157:H7 growth on selective McConkey Sorbitol Agar Base
		Test on b-galactosidase reaction should be (+)	**Test IMVIC** result should be (+)	**Test on oxidase** reaction should be (-)	**Test on sorbitol** reaction should be (-)	**Gram stain performance** reaction should be (-)	
21	+	+	-	-	+	-	-
22	+	-	-	-	+	-	-
23	+	-	-	-	+	-	-
24	+	+	-	-	+	-	-
25	+	-	-	+	+	-	-
101	+	+	-	-	+	-	-
102	+	-	-	-	+	-	-
103	+	-	-	-	+	-	-
104	+	+	-	-	+	-	-
105	+	-	-	-	+	-	-
71	+	+	-	-	+	-	-
72	+	+	+	+	+	-	-
73	+	+	+	-	-	-	+
74	+	-	-	-	+	-	-
75	+	-	-	+	+	-	-

ID	Salmonella growth on selective Bismut Sulfit Agar	Salmonella growth on selective Improved Salmonella Agar	Reactions for salmonella presence								
			Test on b-galactosidase reaction should be (+)	**Test on lactose** reaction should be (-)	**Test on glucose** reaction should be (+)	**Test on sucrose** reaction should be (-)	**Test on indol** reaction should be (-)	**Test on VP** reaction should be (-)	**Test on urease** reaction should be (-)	**Test on H2S** reaction should be (+)	**Gram stain performance** reaction should be (-)
21	-	-	-	+	-	+	+	+	+	-	-
22	-	-	+	+	-	+	+	+	+	-	-
23	+	-	+	+	+	+	+	-	+	+	-
24	-	-	-	+	-	+	+	+	+	-	-
25	+	-	-	+	+	+	+	-	+	+	-
101	-	-	-	+	-	+	+	+	+	-	-
102	-	-	-	+	-	+	+	+	+	-	-
103	-	-	-	+	-	+	+	+	+	-	-
104	+	-	-	+	+	+	+	-	+	+	-
105	+	-	-	+	+	+	+	-	+	+	-
71	-	-	-	+	-	+	+	+	+	-	-
72	+	-	-	+	+	+	+	-	+	+	-
73	-	-	-	+	-	+	+	+	+	-	-
74	-	-	-	+	-	+	+	+	+	-	-
75	-	-	-	+	-	+	+	+	+	-	-

ID	Listeria monocytogenes growth on selective media PALKAM	Reactions for Listeria monocytogenes presence					
		Test on rhamnose reaction should be (-)	**Test on xylose** reaction should be (-)	**Test on manit** reaction should be (-)	**Test on nitrate** reaction should be (-)	**Test on lycitinase activity** reaction should be (+)	**Gram stain performance** reaction should be (+)
21	-	+	+	+	+	-	-
22	-	+	+	+	+	-	-
23	+	+	-	-	+	-	+
24	+	+	-	-	-	-	+
25	+	+	-	+	+	-	+
101	+	+	-	+	+	-	+
102	-	+	+	+	+	-	-
103	-	+	+	+	+	-	-
104	-	+	+	+	+	-	-
105	+	+	-	+	+	-	+
71	+	+	-	+	+	-	+
72	+	+	-	+	+	-	+
73	-	+	-	+	+	-	-
74	-	+	+	+	+	-	-
75	+	+	-	+	+	-	+

ID	Producer	Molds	Total coliforms in Kessler media broth		Total coliforms in BGLB media broth		Performance on Petrifilm in 1ml of 1:10000 dilution (>0 = contaminated)		Performance on Petrifilm in 1ml of 1:1000 dilution (>10 = contaminated)	
			Inoculation from **1:1000** delution	Inoculation from **1:10000** delution	Inoculation from **1:1000** delution	Inoculation from **1:10000** delution	**# of coliform colonies**	**Samples safety for coliforms**	**# of Enterobacter.colonies**	**Samples safety for Enterobact.**
61	Kalinino	yeast	+	-	+	-	1	unsafe	3	safe
62	Sisian	yeast	+	-	+	+	0	safe	41	unsafe
63	Kalinino	yeast	-	-	+	-	0	safe	1	safe
64	Goris	yeast	+	+	+	+	1	unsafe	8	safe
65	Vanadzor	yeast	+	+	+	+	17	unsafe	23	unsafe
121	Goris	yeast	+	+	+	+	2	unsafe	65	unsafe
122	Sisian	yeast	+	+	+	+	34	unsafe	1200	unsafe
123	Kalinino	yeast,Niger,Mucor	+	+	+	+	45	unsafe	500	unsafe
124	Stepanav	yeast	+	+	+	+	>1000	unsafe	>10000	unsafe
125	Kalinino	yeast	+	+	+	+	>1000	unsafe	>10000	unsafe
111	Goris	yeast	+	+	+	+	>1000	unsafe	>10000	unsafe
112	Kalinino	yeast,Aspergillus fumigatus	+	+	+	+	>1000	unsafe	>10000	unsafe
113	Kalinino	yeast	+	+	+	+	13	unsafe	1000	unsafe
114	Sisian	yeast	+	+	+	+	45	unsafe	300	unsafe
115	Kalinino	yeast	+	+	+	+	55	unsafe	600	unsafe

ID	Coliforms and E.coli growth on Endo selective media	Reactions for E.coli presence					E. coli O157:H7 growth on selective McConkey Sorbitol Agar Base
		Test on b-galactosidase reaction should be (+)	**Test IMVIC** result should be (+)	**Test on oxidase** reaction should be (-)	**Test on sorbitol** reaction should be (-)	**Gram stain performance** reaction should be (-)	
61	+	+	-	-	+	-	-
62	+	+	-	-	+	-	-
63	+	-	-	-	+	-	-
64	+	+	-	-	+	-	-
65	+	-	-	+	+	-	-
121	+	-	-	+	+	-	-
122	+	+	-	-	+	-	-
123	+	-	-	-	+	-	-
124	+	-	-	-	+	-	-
125	+	+	-	-	+	-	-
111	+	-	-	-	+	-	-
112	+	+	-	-	+	-	-
113	+	+	-	-	+	-	-
114	+	-	-	-	+	-	-
115	+	-	-	-	+	-	-

ID	Salmonella growth on selective Bismut Sulfit Agar	Salmonella growth on selective Improved Salmonella Agar	Reactions for salmonella presence								
			Test on b-galactosidase reaction should be (+)	**Test on lactose** reaction should be (-)	**Test on glucose** reaction should be (+)	**Test on sucrose** reaction should be (-)	**Test on indol** reaction should be (-)	**Test on VP** reaction should be (-)	**Test on urease** reaction should be (-)	**Test on H2S** reaction should be (+)	**Gram stain performance** reaction should be (-)
61	-	-	-	+	-	+	+	+	+	-	-
62	+	-	-	+	+	+	+	-	+	+	-
63	+	-	-	+	+	+	+	-	+	+	-
64	+	-	-	+	+	+	+	-	+	+	-
65	+	-	-	+	+	+	+	-	+	+	-
121	-	-	-	+	-	+	+	+	+	-	-
122	-	-	+	+	-	+	+	+	+	-	-
123	-	-	-	+	-	+	+	+	+	-	-
124	-	-	-	+	-	+	+	+	+	-	-
125	+	-	-	+	+	+	+	-	+	+	-
111	-	-	-	+	-	+	+	+	+	-	-
112	+	-	-	+	+	+	+	-	+	+	-
113	-	-	+	+	-	+	+	+	+	-	-
114	-	-	-	+	-	+	+	+	+	-	-
115	+	-	-	+	+	+	+	-	+	+	-

ID	Listeria monocytogenes growth on selective media PALKAM	Reactions for Listeria monocytogenes presence					
		Test on rhamnose reaction should be (-)	**Test on xylose** reaction should be (-)	**Test on manit** reaction should be (-)	**Test on nitrate** reaction should be (-)	**Test on lycitinase activity** reaction should be (+)	**Gram stain performance** reaction should be (+)
61	+	+	+	+	+	-	+
62	-	+	+	+	+	-	-
63	-	+	+	+	+	-	-
64	+	+	-	+	+	-	+
65	+	+	-	+	+	-	+
121	+	+	-	+	+	-	+
122	+	+	-	+	+	-	+
123	+	+	-	-	-	-	+
124	+	+	-	-	-	+	+
125	+	+	-	+	+	-	+
111	+	+	-	+	+	-	+
112	-	+	+	+	+	-	-
113	-	+	+	+	+	-	-
114	-	+	+	+	+	-	-
115	-	+	+	+	+	-	-