

## Culture Media for Accelerated Isolation of Listeria

*Sagdullaeva G. U.*

Bukhara State Medical University

### Abstract

The article provides data on the survival of listeria in milk, the period of generation of listeria in milk and milk products when stored in a domestic refrigerator for 1, 3 days is studied, and various nutrient media for the accelerated isolation of listeria from milk are studied in comparative terms.

### Keywords:

 Listeria

monocytogenes, mononuclear leukocytosis, psychrophilicity, tellurite-florimycin-chloride medium; Listeria agar cultures, optical turbidity standard.

**Relevance.** Listeriosis is a contagious bacterial infection that affects animals and humans. In humans, the disease is caused by the species *Listeria monocytogenes* - spontaneous and experimental infection is accompanied by mononuclear leukocytosis. The disease occurs in most cases with damage to the nervous tissue or in the form of an anginal-septic form.

The pathogen is excreted from the body of a sick animal in urine, feces, nasal discharge, milk and amniotic fluid (1). In humans, the pathogen was isolated (7) from the cerebrospinal fluid of a patient with meningitis and listeria encephalitis.

A particular danger of listeriosis has arisen due to the established contamination of food products with listeria (3). Large outbreaks of listeriosis have been reported in humans with high mortality rates from the consumption of foods of animal origin (milk, milk products, cheeses, ice cream (2)). These cases have highlighted the importance of food production and consumption due to the possible contamination of food with listeria. In connection with This is why listeriosis received the name “food infection.” Currently, listeria is found in food products, significant resistance of listeria in the external environment, the difficulty of clinical and laboratory diagnosis requires improvement of indication methods, isolation of listeria from pasteurized and fresh milk, as well as studying the survival time of listeria in milk and dairy products (4).

Currently, the problem of foodborne listeriosis is becoming more and more urgent. Purpose of the work: to study the period of generation of listeria in milk to clarify the role of milk and dairy products in the transmission of listeria infection, as well as to study in a comparative aspect nutrient media for isolating listeria from milk.

**Materials and methods.** The studies were carried out with the following strains of *Listeria* cultures: 9-72, 9-127, 2795/5. To study the generation period and survival times of *Listeria* in fresh and pasteurized milk, milk contaminated with *Listeria* was incubated at different temperature conditions: in a thermostat at 35 °C, 45 °C, 50 °C, in a household refrigerator at: +1-2 °C, -0.1 to 0.4 °C. Every 30, 40, 60 minutes, seedings were done on various nutrient media.

Growth on nutrient media after sowing was observed slowly, indicating that *Listeria* is a slow-growing bacteria, doubling time of their numbers at 35°C, 45°C, 50°C (milk) - 41 minutes. In sensitive people, listeriosis can be caused by as few as 100 *L. monocytogenes* cells (5), so even a short period of storing a food product with *Listeria* at the temperature of a household refrigerator for 1.5-3 days can make the product hazardous to health.

Microorganisms found in fresh unpasteurized milk are capable of inhibiting the proliferation of *Listeria*; therefore, the number of *Listeria*, for example, in fresh and chilled milk is no more than 100 cells per 1 g. However, specific conditions during pasteurization help to inhibit the growth of foreign microorganisms and *Listeria* multiply unhindered

An important circumstance in the study of the epizootology of listeriosis is the psychrophilic nature of *Listeria*. We studied the dynamics of *Listeria* survival in fresh and pasteurized milk at the following temperature conditions - +4 °C and +20 °C.

*Listeria* actively multiplied in milk and their maximum number was observed on days 4-5 at +4 °C and on day 4 at +20 °C. The most intensive reproduction of *Listeria* was noted at +4 °C, which is explained by the psychrophilicity of *Listeria*. This fact is also confirmed by numerous outbreaks of listeriosis associated with the consumption of dairy products stored in refrigerators.

To isolate *Listeria* on nutrient media, the effectiveness of a number of nutrient media for isolating *Listeria* from milk was studied in a comparative aspect (Table 1).

The following culture media were tested: tellurite-florimycin-chloride media; Martin's agar; meat peptone agar with potassium tellurite; meat-peptone liver agar with 1% glucose and 2% glycerol; meat peptone agar; indicator medium with bromothymol blue, for the preparation of which 60 ml of indicator 2% aqueous solution of bromothymol blue was added to 1000 ml of MPPA, the initial color of the medium is dark green, the causative agent of listeriosis changes it to yellow as it grows; *Listeria* colonies in the first 24 hours of growth are smoky-gray in color, and after 36-48 hours they become golden-yellow with a characteristic morphology; indicator medium with bromocresol purple indicator (add 60 ml of 2% solution of bromocresol purple indicator per 1000 ml of MPPA); meat-peptone broth with the addition of 8.5% sodium chloride; MPPB with potassium thiocyanate; MPB. From daily agar cultures of *Listeria* strains 9-72, 9-127, 2795/5, a suspension was prepared

containing 1 billion microbial cells in 1 ml according to the optical turbidity standard. After microscopy and examination of the agglutination reaction, dilutions were made in sterile and fresh unpasteurized milk from 1:100 to 1:100,000. 0.1 ml of each dilution was applied to the surface of the medium in Petri dishes and incubated at 37°C. When cultivated on liquid nutrient media, they were subsequently reseeded onto solid media and the presence of colonies, their number, morphology, etc. was determined.

Intensive growth was observed near the surface of the medium, which indicated the tendency of *Listeria* to accelerate growth at oxygen potential levels lower than that in the air. Crops on solid media were viewed in transmitted light using an OI-31 illuminator with a blue filter. Colonies of *Listeria* in transmitted light, unlike colonies of other microorganisms, have a blue color with a greenish tint and a fine-grained structure, have the smell of cottage cheese or whey, due to the accumulation of carbohydrate metabolism products. In addition, smears were prepared and Gram stained. For control, pure cultures of *Listeria* were examined using the same tests.

The effectiveness of the medium was assessed by the growth time of *Listeria* and the number of colonies, taking into account the dilution of the test material.

The most effective for isolating *Listeria* from artificially infected milk were: from dense milk - tellurite-florimitsin-chloride medium, MPA with potassium tellurite; from liquid - MPPB with potassium thiocyanate and MPB with 8.5% sodium chloride (see table)

On the tellurite-florimycin-chloride medium, growth in the form of tiny colonies was observed on the first day; on the second day, they acquired a black color due to the reduction of potassium tellurite to metallic tellurium.

On liquid media - MPPB with potassium rhodanate and MPB with the addition of 8.5% sodium chloride - growth manifested itself in the form of barely noticeable opalescence. When sown on these media at a dilution of 1:100,000, *Listeria* grew in 100% of cases; when other liquid nutrient media were used for this purpose, growth in 100% of cases occurred only when sown at a dilution of 1:100,000 or less.

**Table 1. EFFECTIVENESS OF A SERIES OF NUTRIENT MEDIA FOR ISOLATION OF LISTERIA FROM MILK**

nutrient media	1:100	1:1000	1:10000	1:1000000
Tellurite-florimycin-chloride medium	740 ± 23,2	80 ± 5,2	12 ± 1,5	1
MPPA with 0.5% glucose and 1% glycerol	420 ± 33,2	30 ± 12,9	3 ± 1,4	-
Martin's agar	640 ± 11,6	45 ± 15,8	7 ± 1,2	-

Medium with bromothymolblue	650 ±17,4	30 ±2,9	8 ±1,2	-
Medium with bromocresol purple	400 ±40,6	25 ±1,7	7 ±1,2	-
MPA with potassium tellurite	800 ±62,8	70 ±5,8	10 ±2,6	1
MPPB with potassium thiocyanate	500 ±19,3	75 ±2,9	10 ±2,6	1
MPB with 8.5% sodium chloride	600 ±11,6	60 ±2,9	7 ±1,1	1
MPB	300 ±11,6	40 ±2,9	2 ±5,8	-
IPA	410 ±17,4	50 ±1,7	3 ±1,4	-

### Conclusion.

1. The maximum amount of listeria in milk was observed on days 4-5 at +4 °C and on day 4 at +20 °C.
2. The most effective for isolating Listeria from milk were tellurite-florimitsin-chloride medium, MPA with potassium tellurite from solid nutrient media; MPB with the addition of 8.5% sodium chloride.

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