

No. 18095487001-0301

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Date issued: October 08, 2018

REPORT

Client: FLAX Co., Ltd.
4-1 Hommokumotomachi, Naka-ku, Yokohama-shi, Kanagawa 231-0822, Japan

Sample(s): Hypochlorous acid water Generation bottle 「ZIA pocket」

Title: Bacterial Elimination Ability Confirmation Test of Generated Water

Received date of sample(s): August 21, 2018

This report has been translated into English from Japanese report No. 18095487001-0101 (Date issued: October 08, 2018).

Signed for and on behalf of JFRL



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Section of Analysis Documentation

Dec. 14, 2018

Date

Bacterial Elimination Ability Confirmation Test of Generated Water

1. Client

FLAX Co., Ltd.

2. Sample

Hypochlorous acid water Generation bottle 「ZIA pocket」
Sodium chloride was provided by the client.

3. Outline of the method

Purified water and sodium chloride were added to the sample and mixed. The sample was then allowed to operate and the generated water was used as the test water.

The test cell suspension was inoculated to the test water (hereafter called "the test solution"). At a designated measurement point, the viable cell count of the test solution was measured.

A preliminary test (confirmation of the conditions for neutralization) was performed to determine the test conditions in which the viable cell count measurement could be performed without the effect of the sample.

4. Results

Table 1 shows the test results and Table 2 lists the test conditions. Photos 1 to 40 show the agar plates after incubation.

Based on the results of the preliminary test (shown in Table 2, Neutralization conditions), it was confirmed that the test solution should be diluted with SCDLP broth (SCDLP broth containing 3 % sodium chloride for *Vibrio parahaemolyticus*) so that the viable cell count measurement could be performed without the effect of the sample.

Table 1. Viable cell counts of test solutions

Test organism	Test specimen	Viable cell count (per mL)			
		Initial	After 30 sec.	After 1 min.	After 5 min.
<i>Escherichia coli</i> (O157:H7)	Test water	—	<10	<10	<10
	Control (1)	—	5.0×10^5	4.6×10^5	3.9×10^5
	Control (2)	5.8×10^5	—	—	5.1×10^5
<i>Salmonella enterica</i>	Test water	—	<10	<10	<10
	Control (1)	—	6.4×10^5	6.0×10^5	7.0×10^5
	Control (2)	7.2×10^5	—	—	7.3×10^5
<i>Staphylococcus aureus</i>	Test water	—	<10	<10	<10
	Control (1)	—	4.7×10^5	4.6×10^5	3.8×10^5
	Control (2)	4.8×10^5	—	—	5.4×10^5
<i>Vibrio parahaemolyticus</i>	Test water	—	<10	<10	<10
	Control (1)	—	1.8×10^2	<10	<10
	Control (2)	1.8×10^5	—	—	2.1×10^5
<i>Aspergillus niger</i>	Test water	—	3.6×10^5	8.8×10^4	20
	Control (1)	—	1.3×10^5	4.0×10^5	2.0×10^5
	Control (2)	1.8×10^5	—	—	1.5×10^5

Test water: 180 mL of purified water and 1 g of sodium chloride were added to the sample and mixed.

The sample was then allowed to operate for 3 minutes.

Control (1): 1 g of sodium chloride was added to 180 mL of purified water.

Control (2): Purified water (physiological saline for *Staphylococcus aureus*, 3 % sodium chloride solution for *Vibrio parahaemolyticus*)

Storage temperature: Room temperature

<10: Not detected

Table 2-1. Test conditions

Test cell suspension	Test organisms	(1) <i>Escherichia coli</i> ATCC 43895 (Serotype O157:H7, Produces Shiga-like toxins I & II) (2) <i>Salmonella enterica</i> subsp. <i>enterica</i> NBRC 3313 (3) <i>Staphylococcus aureus</i> subsp. <i>aureus</i> NBRC 12732 (4) <i>Vibrio parahaemolyticus</i> RIMD 2210100 (5) <i>Aspergillus niger</i> NBRC 105649
	Test organisms (1) and (2) Incubation conditions: Nutrient Agar (Eiken Chemical Co., Ltd.) at 35 °C ± 1 °C for 18 to 24 hours Solution of test cell suspension: Purified water Concentration of test cell suspension: 10 ⁷ -10 ⁸ /mL	
	Test organism (3) Incubation conditions: Nutrient Agar at 35 °C ± 1 °C for 18 to 24 hours Solution of test cell suspension: Physiological saline Concentration of test cell suspension: 10 ⁷ -10 ⁸ /mL	
	Test organism (4) Incubation conditions: Nutrient Agar containing 3 % sodium chloride at 35 °C ± 1 °C for 18 to 24 hours Solution of test cell suspension: 3 % sodium chloride solution Concentration of test cell suspension: 10 ⁷ -10 ⁸ /mL	
	Test organism (5) Incubation conditions: Potato Dextrose Agar (Difco) at 25 °C ± 1 °C for 7 to 10 days Solution of test cell suspension: 0.005 % dioctyl sodium sulfosuccinate solution Concentration of test cell suspension: 10 ⁷ -10 ⁸ /mL	
Test water	180 mL of purified water and 1 g of sodium chloride were added to the sample and mixed. The sample was then allowed to operate for 3 minutes.	
Test solution	0.1 mL of the test cell suspension was inoculated to 10 mL of the test water.	
Storage conditions	Time: 30 seconds, 1 and 5 minutes Temperature: Room temperature	
Control (1)	1 g of sodium chloride was added to 180 mL of purified water.	
Control (2)	Test organisms (1), (2) and (5): Purified water Test organism (3): Physiological saline Test organism (4): 3 % sodium chloride solution	
Neutralization conditions	Test organisms (1)-(3) and (5): The test solution was diluted 10-fold with SCDLP broth (Nihon Pharmaceutical Co., Ltd.). Test organism (4): The test solution was diluted 10-fold with SCDLP broth containing 3 % sodium chloride.	

Table 2-2. Test conditions

Measurement of viable cell counts	Test organisms (1)-(3): SCDLP agar (Nihon Pharmaceutical Co., Ltd.) Pour plate method	Incubation conditions: 35 °C ± 1 °C 2 days
	Test organism (4): SCDLP agar containing 3 % sodium chloride Pour plate method	Incubation conditions: 35 °C ± 1 °C 2 days
	Test organism (5): GPLP agar (Nihon Pharmaceutical Co., Ltd.) Pour plate method	Incubation conditions: 25 °C ± 1 °C 7 days

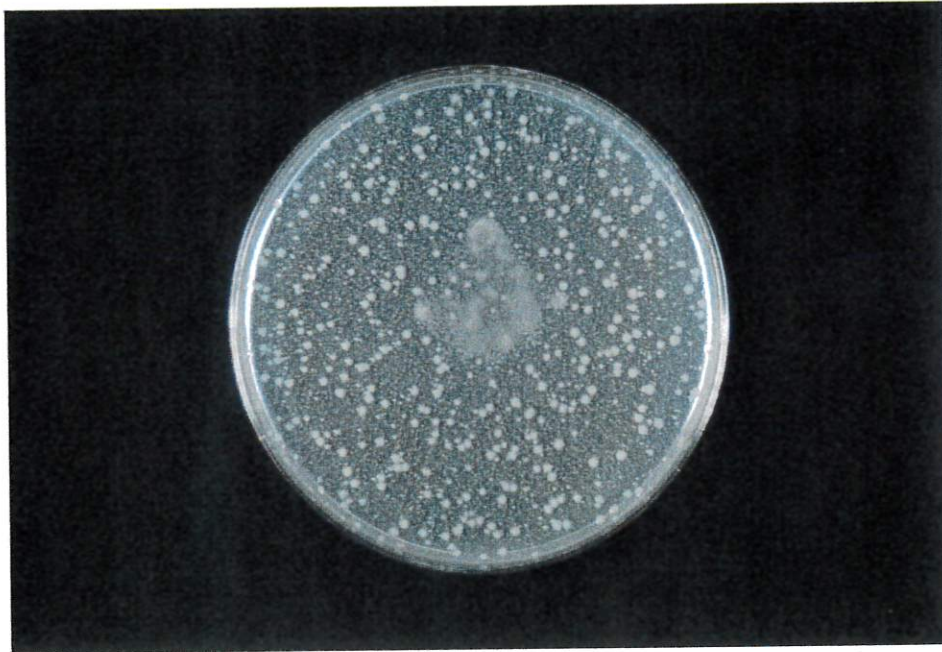


Photo. 1: *Escherichia coli* (O157:H7), Control (2), Initial
(0.1 mL of the test solution)

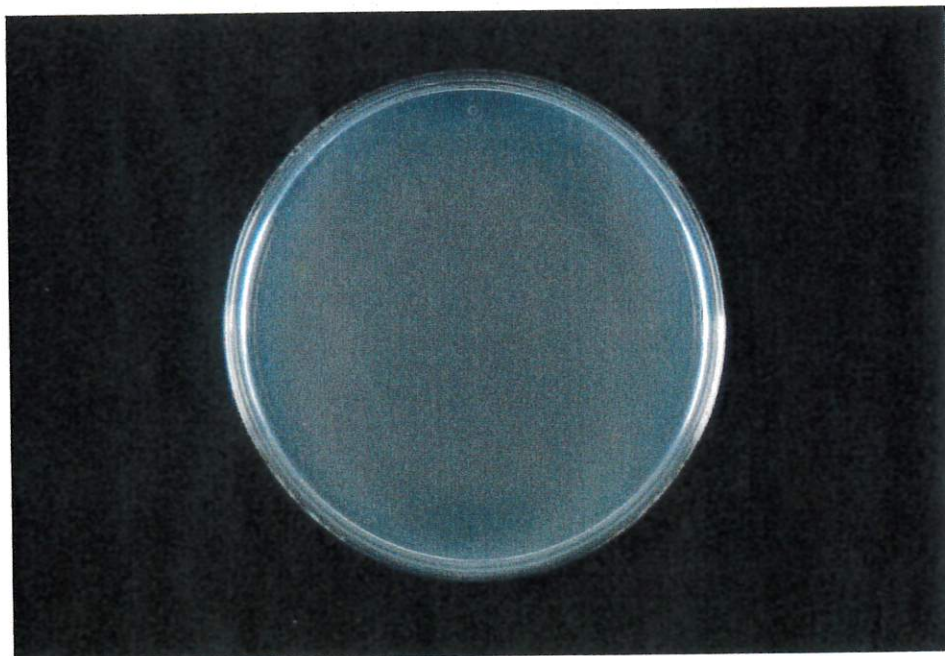


Photo. 2: *Escherichia coli* (O157:H7), Test water, After 30 seconds
(0.1 mL of the test solution)

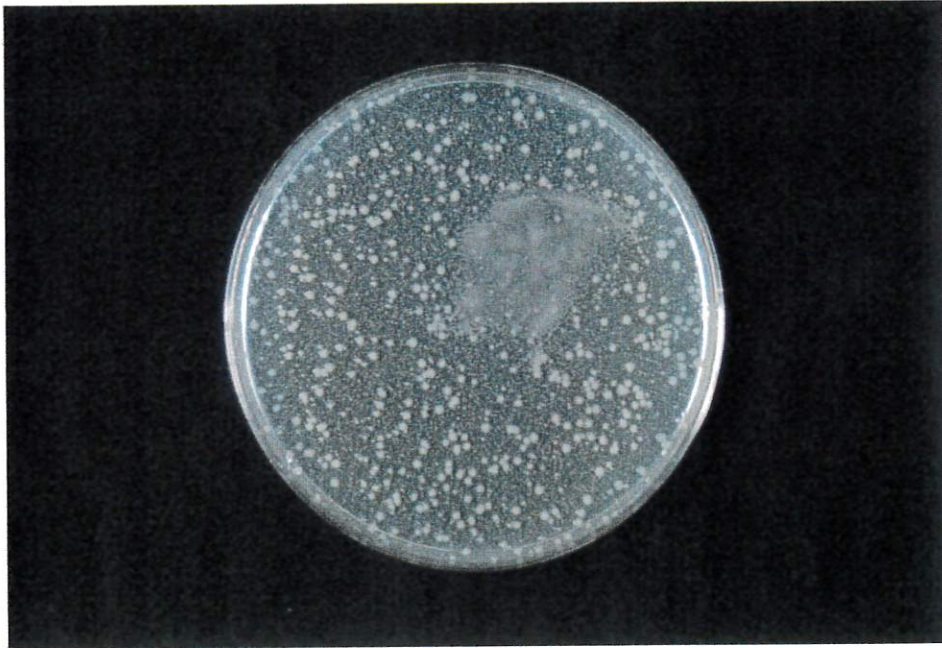


Photo. 3: *Escherichia coli* (O157:H7), Control (1), After 30 seconds
(0.1 mL of the test solution)

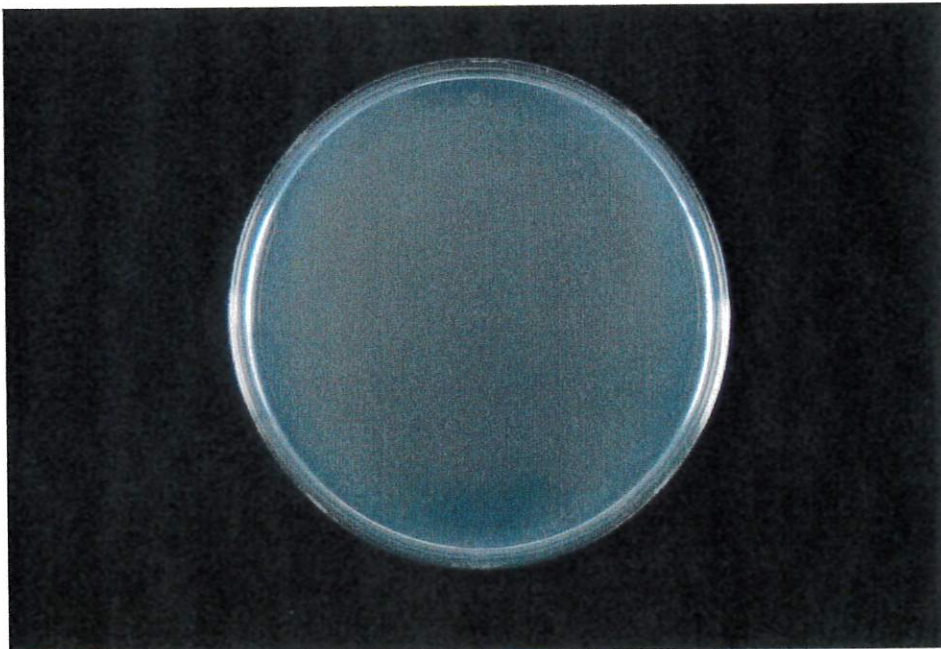


Photo. 4: *Escherichia coli* (O157:H7), Test water, After 1 minute
(0.1 mL of the test solution)

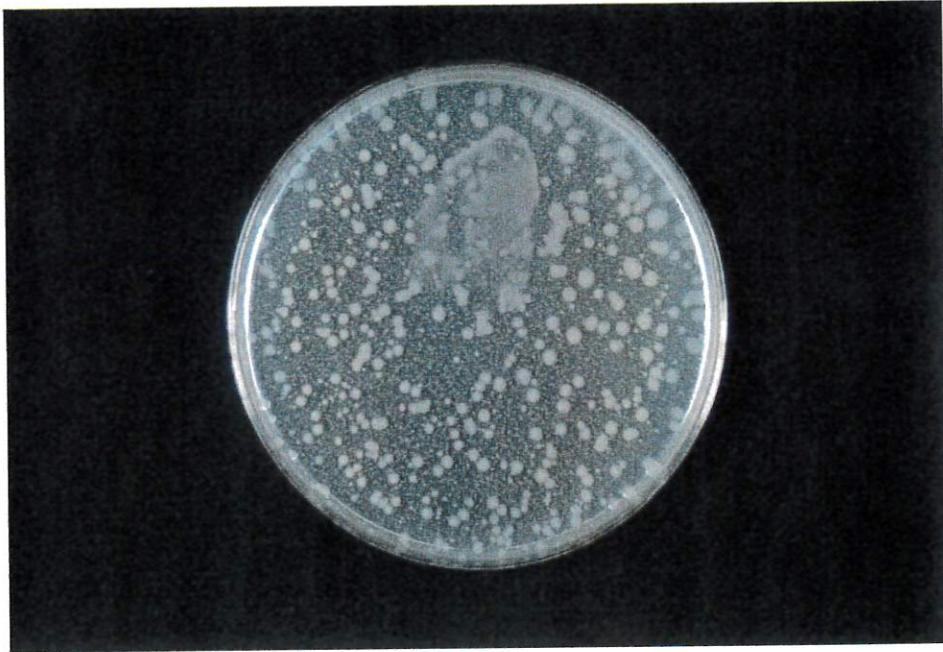


Photo. 5: *Escherichia coli* (O157:H7), Control (1), After 1 minute
(0.1 mL of the test solution)

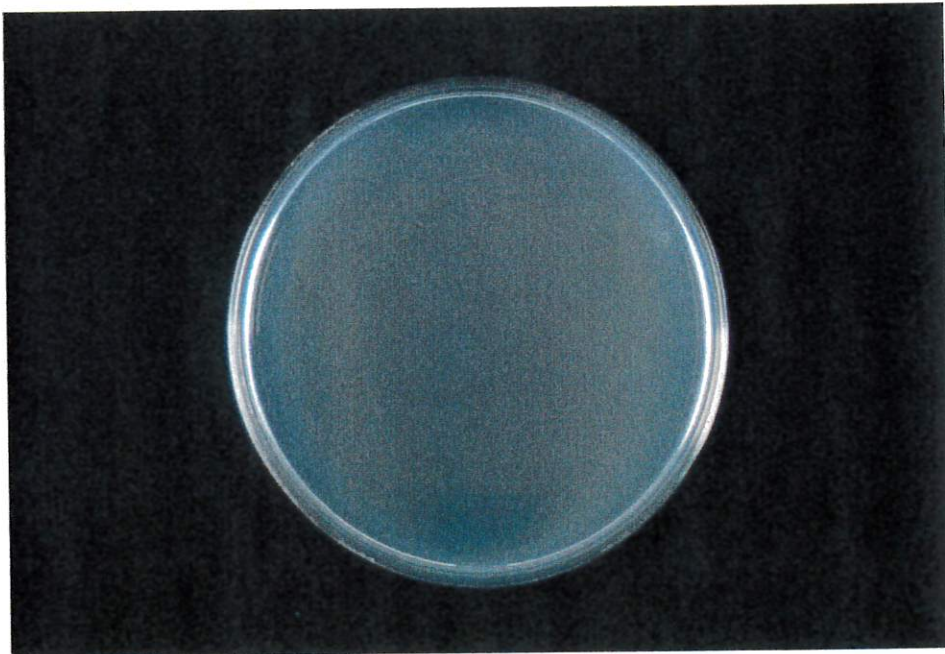


Photo. 6: *Escherichia coli* (O157:H7), Test water, After 5 minutes
(0.1 mL of the test solution)

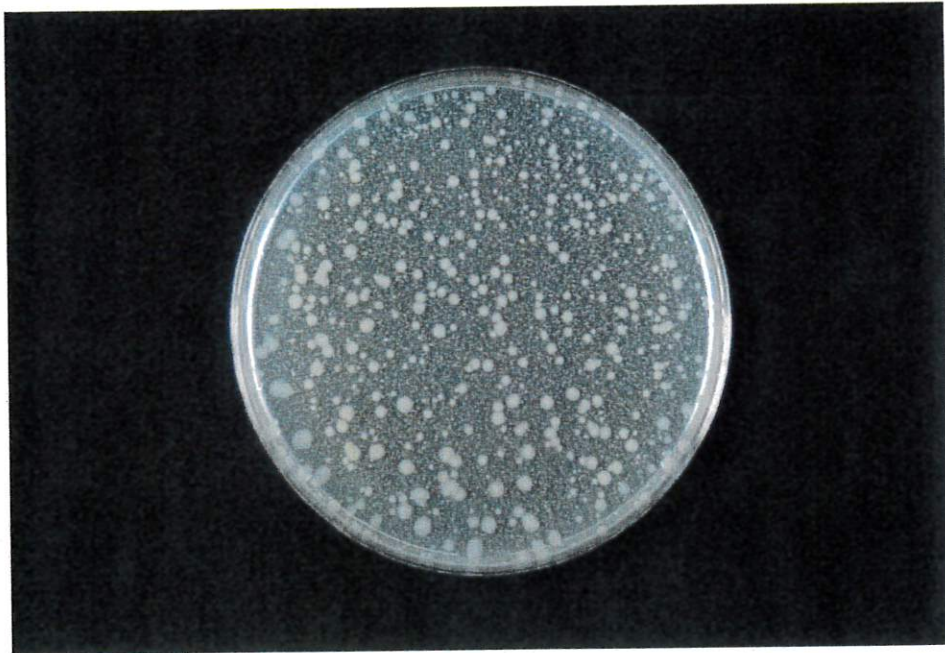


Photo. 7: *Escherichia coli* (O157:H7), Control (1), After 5 minutes
(0.1 mL of the test solution)

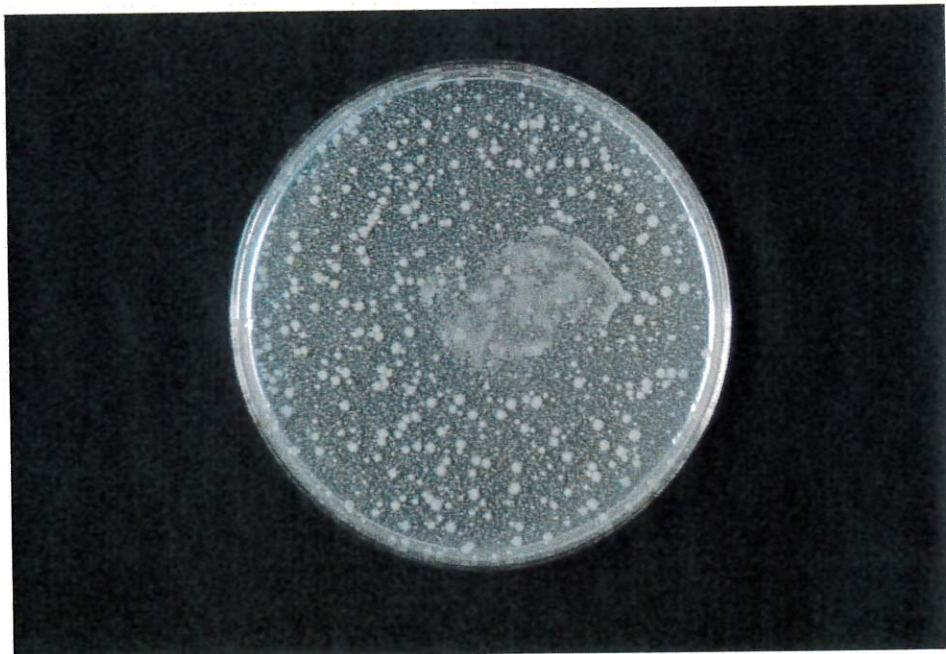


Photo. 8: *Escherichia coli* (O157:H7), Control (2), After 5 minutes
(0.1 mL of the test solution)

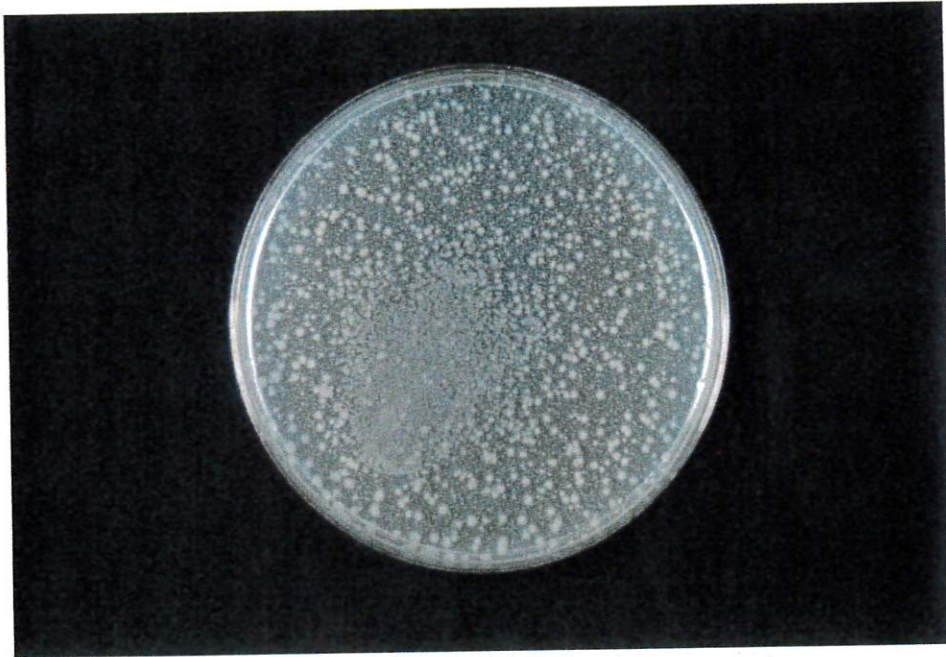


Photo. 9: *Salmonella enterica*, Control (2), Initial
(0.1 mL of the test solution)

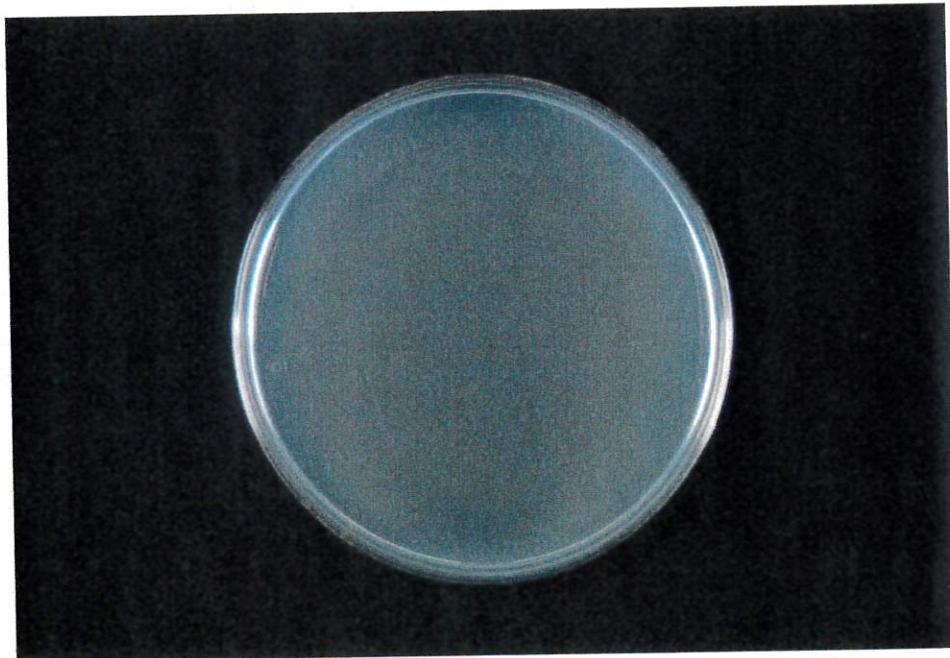


Photo. 10: *Salmonella enterica*, Test water, After 30 seconds
(0.1 mL of the test solution)

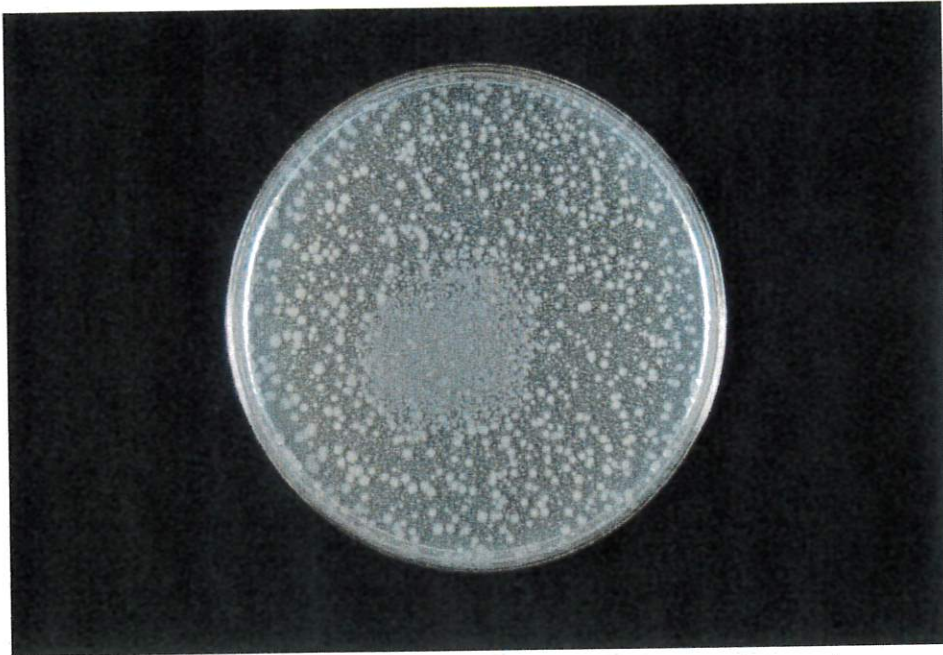


Photo. 11: *Salmonella enterica*, Control (1), After 30 seconds
(0.1 mL of the test solution)

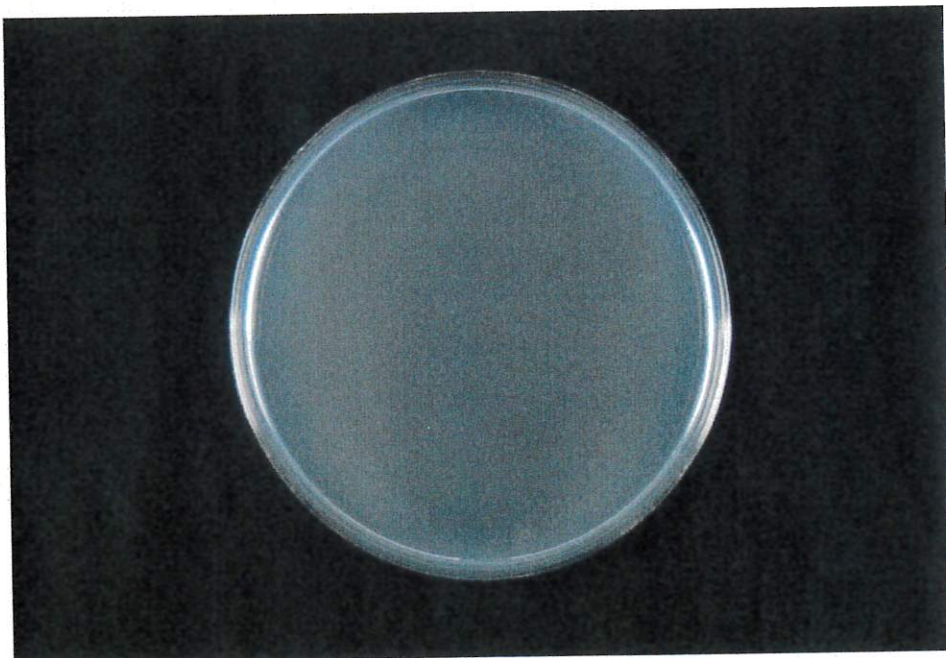


Photo. 12: *Salmonella enterica*, Test water, After 1 minute
(0.1 mL of the test solution)



Photo. 13: *Salmonella enterica*, Control (1), After 1 minute
(0.1 mL of the test solution)

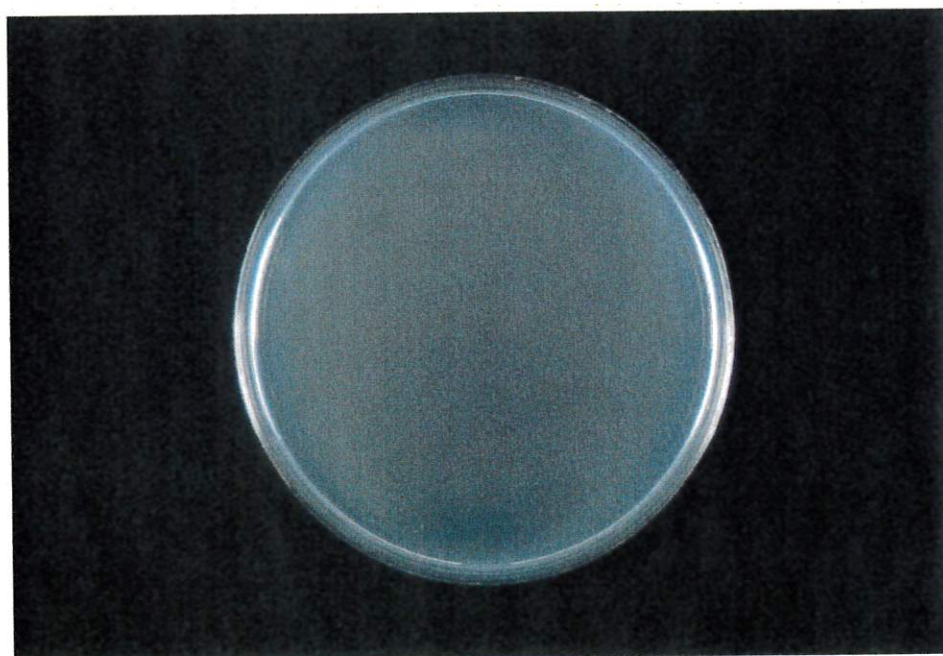


Photo. 14: *Salmonella enterica*, Test water, After 5 minutes
(0.1 mL of the test solution)



Photo. 15: *Salmonella enterica*, Control (1), After 5 minutes
(0.1 mL of the test solution)

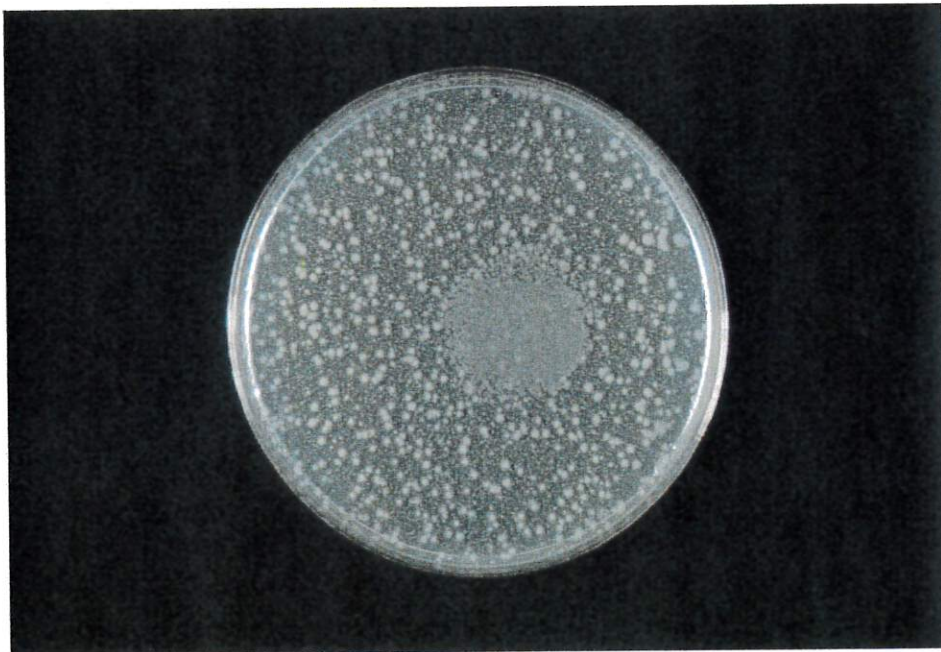


Photo. 16: *Salmonella enterica*, Control (2), After 5 minutes
(0.1 mL of the test solution)

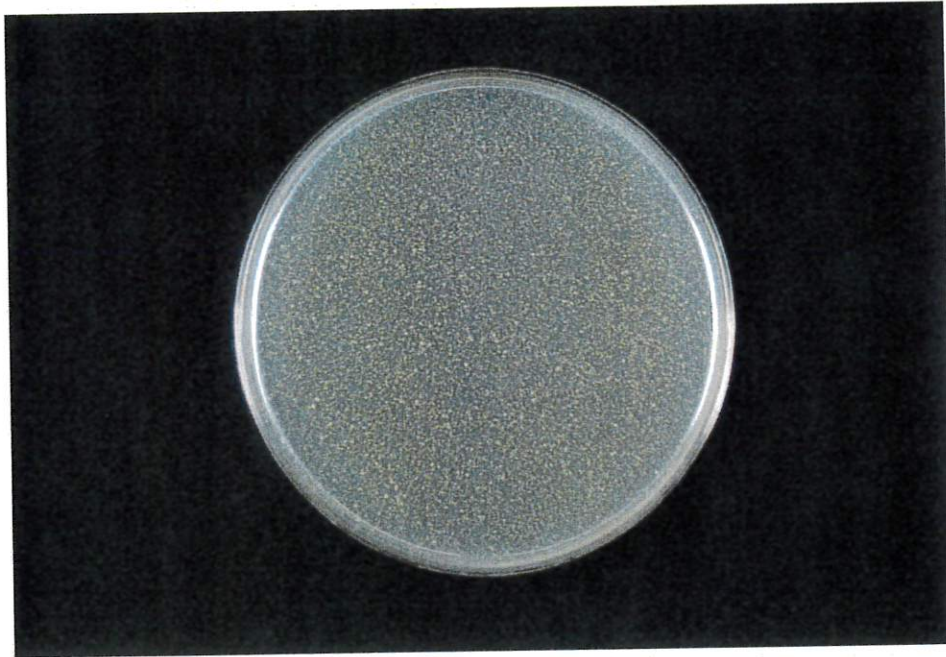


Photo. 17: *Staphylococcus aureus*, Control (2), Initial
(0.1 mL of the test solution)

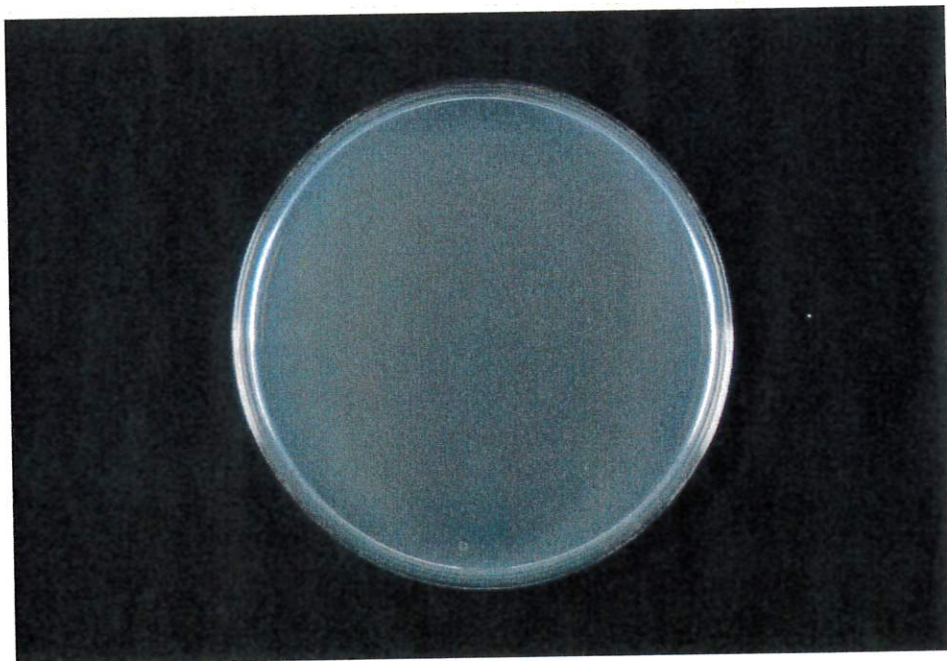


Photo. 18: *Staphylococcus aureus*, Test water, After 30 seconds
(0.1 mL of the test solution)

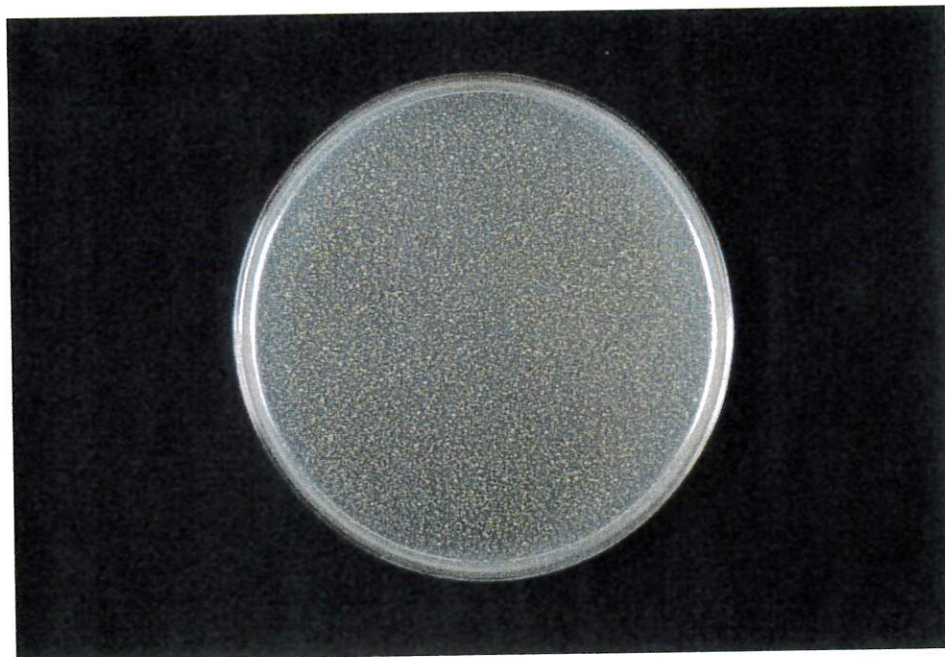


Photo. 19: *Staphylococcus aureus*, Control (1), After 30 seconds
(0.1 mL of the test solution)

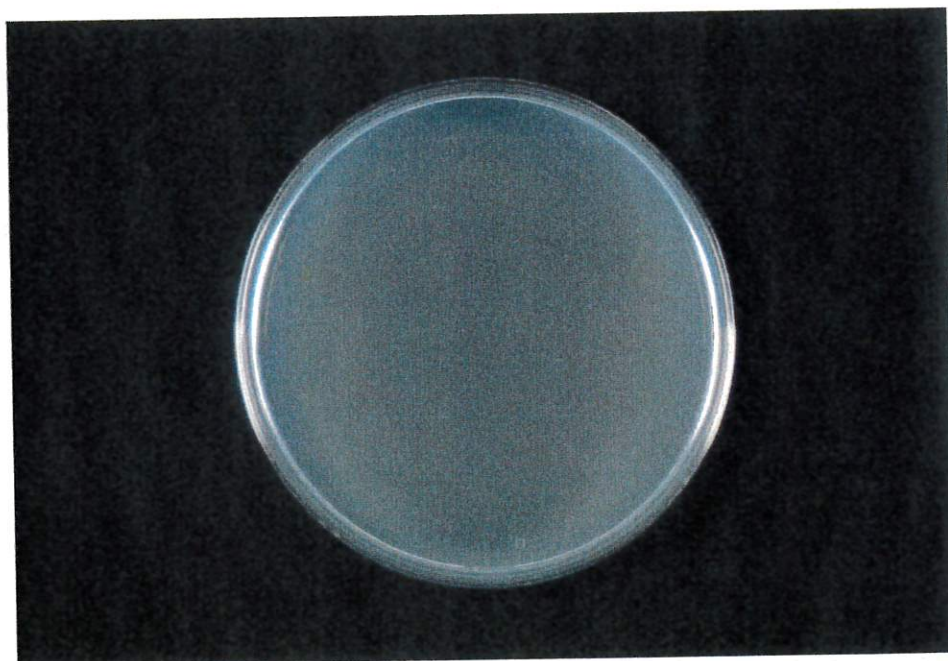


Photo. 20: *Staphylococcus aureus*, Test water, After 1 minute
(0.1 mL of the test solution)

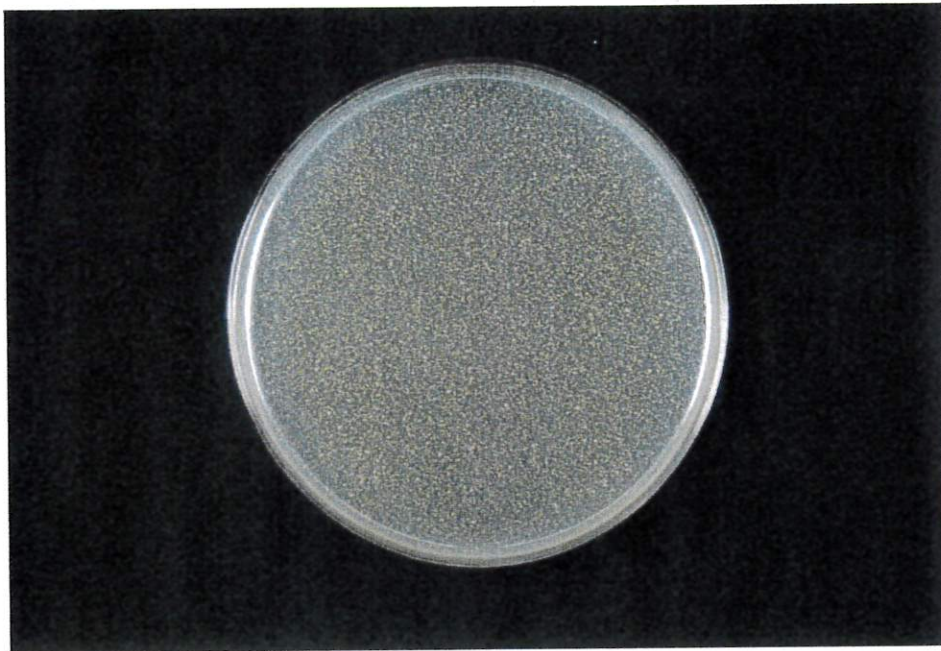


Photo. 21: *Staphylococcus aureus*, Control (1), After 1 minute
(0.1 mL of the test solution)

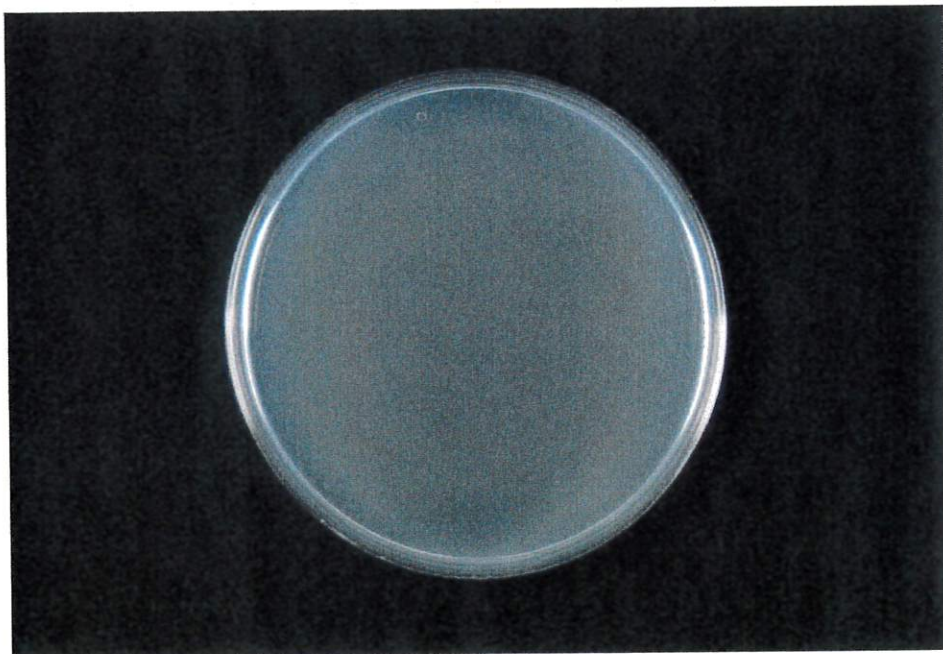


Photo. 22: *Staphylococcus aureus*, Test water, After 5 minutes
(0.1 mL of the test solution)



Photo. 23: *Staphylococcus aureus*, Control (1), After 5 minutes
(0.1 mL of the test solution)

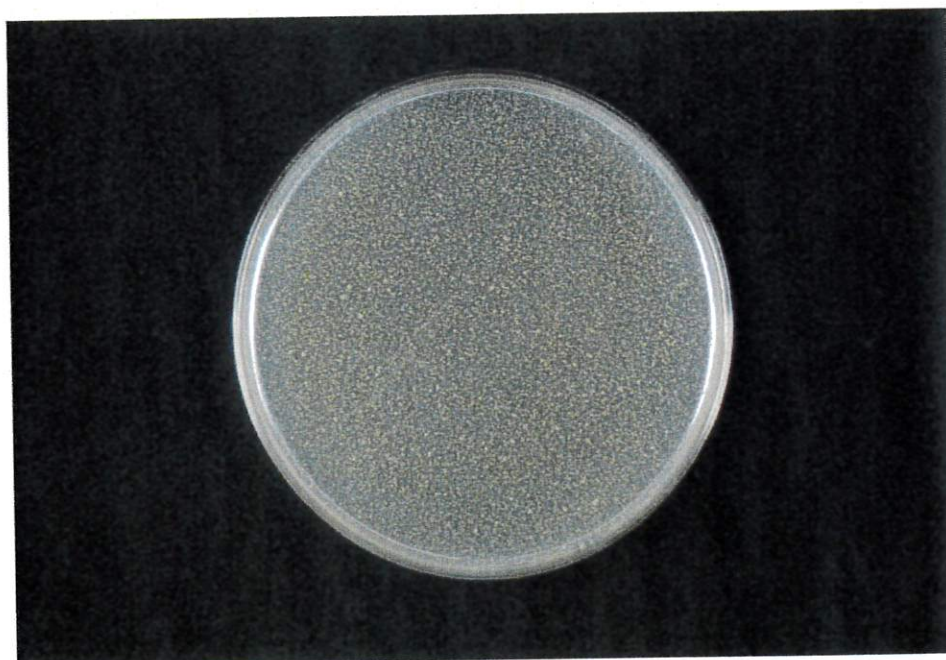


Photo. 24: *Staphylococcus aureus*, Control (2), After 5 minutes
(0.1 mL of the test solution)



Photo. 25: *Vibrio parahaemolyticus*, Control (2), Initial
(0.1 mL of the test solution)

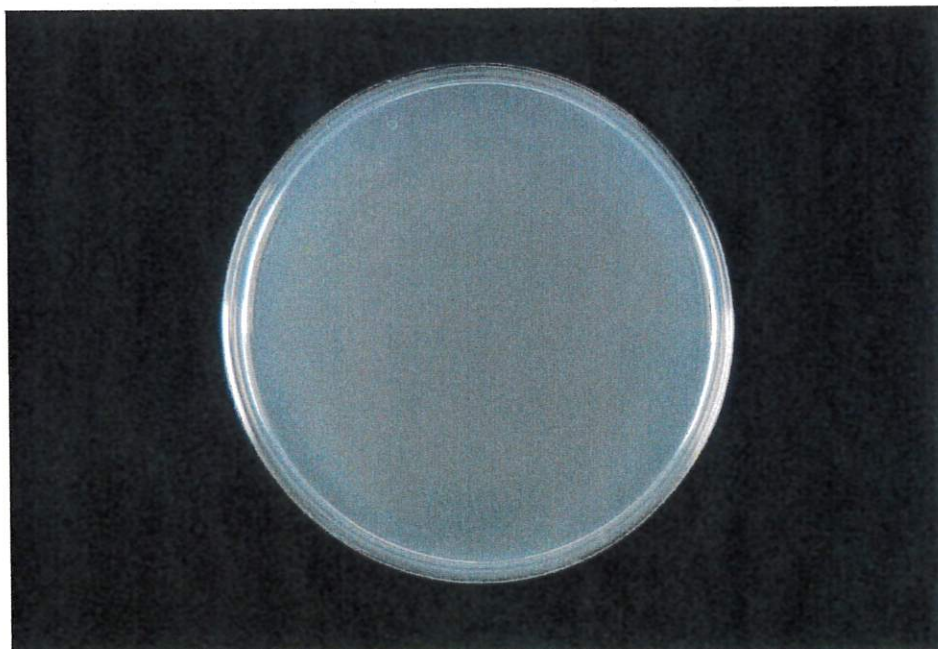


Photo. 26: *Vibrio parahaemolyticus*, Test water, After 30 seconds
(0.1 mL of the test solution)



Photo. 27: *Vibrio parahaemolyticus*, Control (1), After 30 seconds
(0.1 mL of the test solution)

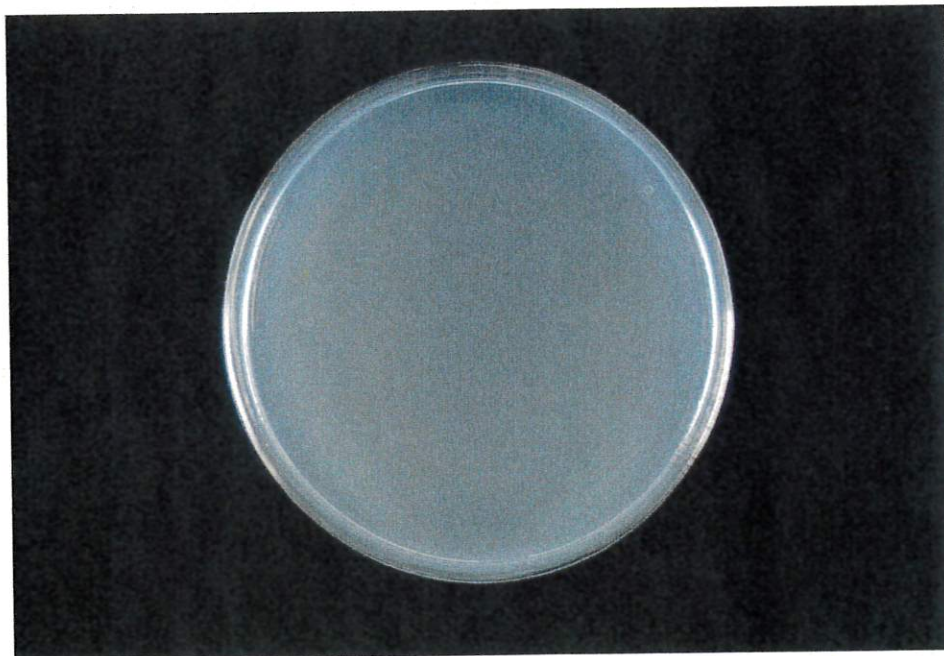


Photo. 28: *Vibrio parahaemolyticus*, Test water, After 1 minute
(0.1 mL of the test solution)

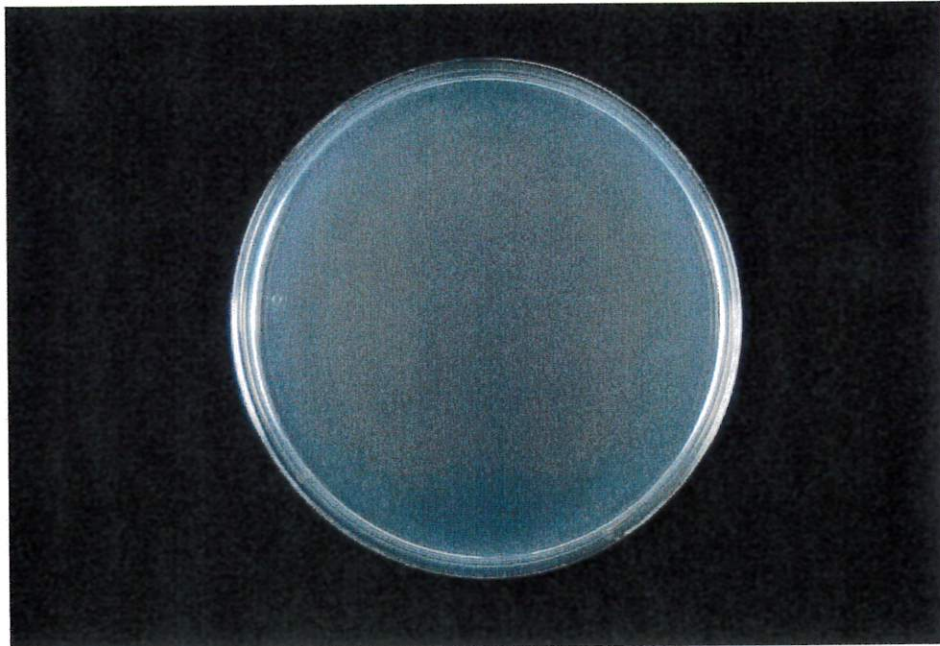


Photo. 29: *Vibrio parahaemolyticus*, Control (1), After 1 minute
(0.1 mL of the test solution)

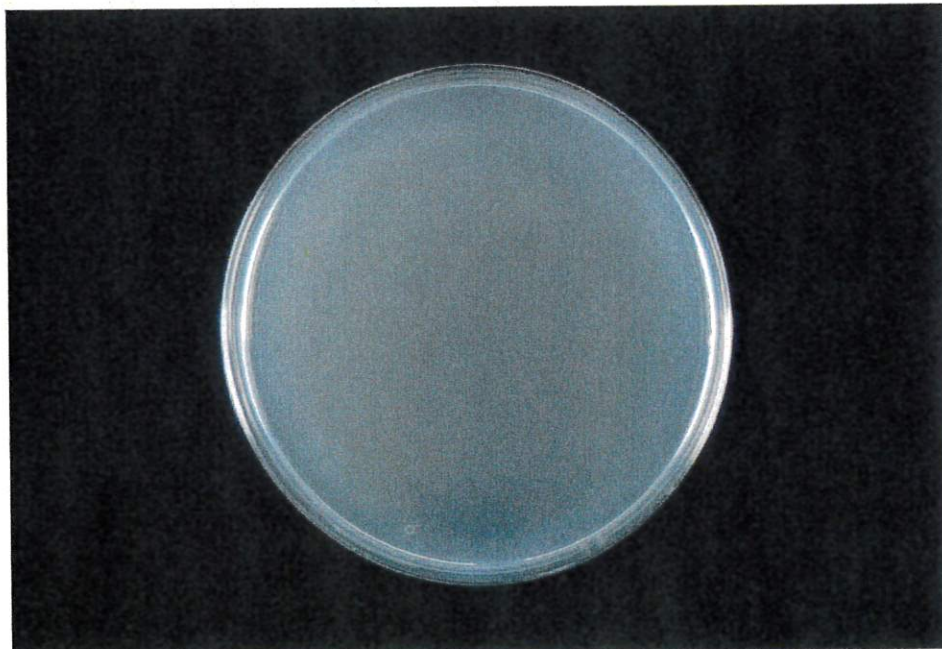


Photo. 30: *Vibrio parahaemolyticus*, Test water, After 5 minutes
(0.1 mL of the test solution)

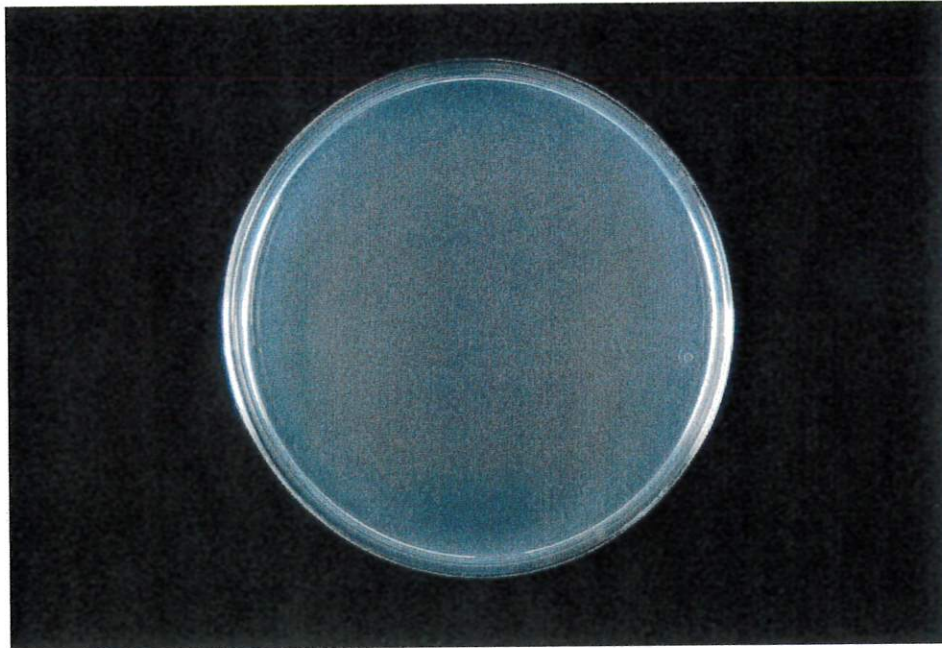


Photo. 31: *Vibrio parahaemolyticus*, Control (1), After 5 minutes
(0.1 mL of the test solution)



Photo. 32: *Vibrio parahaemolyticus*, Control (2), After 5 minutes
(0.1 mL of the test solution)

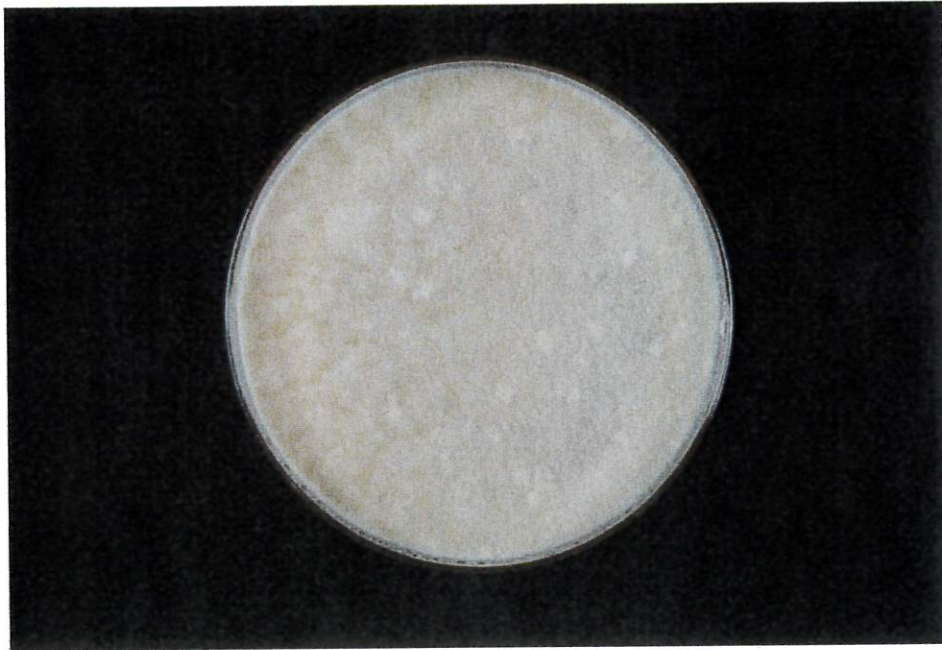


Photo. 33: *Aspergillus niger*, Control (2), Initial
(0.1 mL of the test solution)

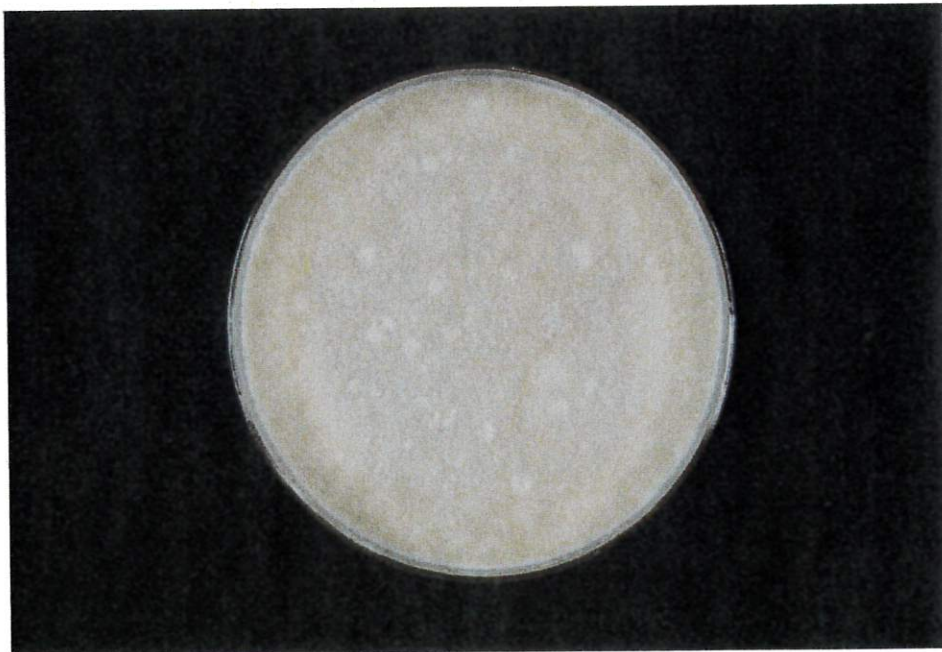


Photo. 34: *Aspergillus niger*, Test water, After 30 seconds
(0.1 mL of the test solution)



Photo. 35: *Aspergillus niger*, Control (1), After 30 seconds
(0.1 mL of the test solution)

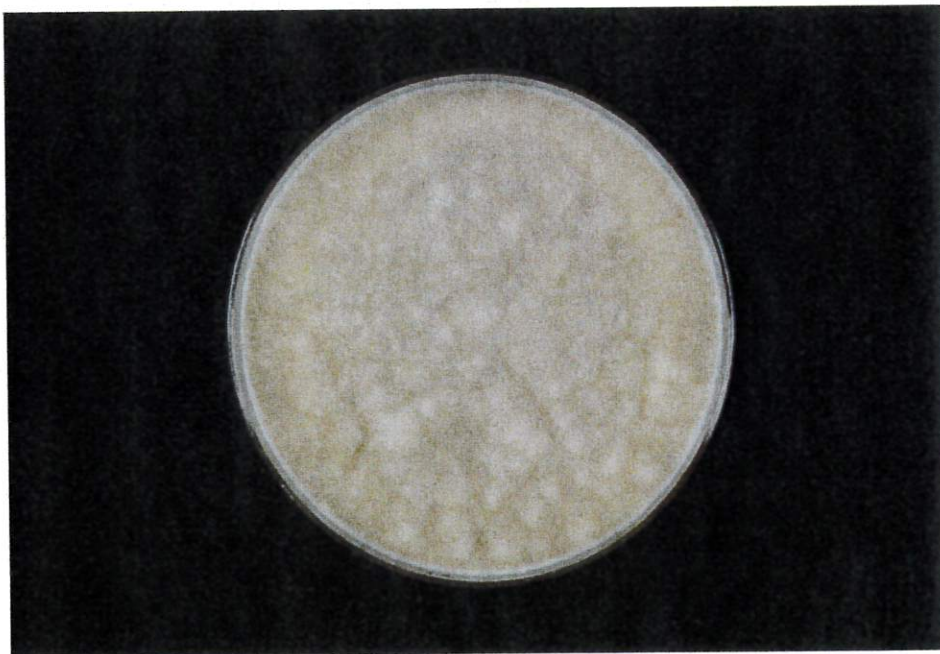


Photo. 36: *Aspergillus niger*, Test water, After 1 minute
(0.1 mL of the test solution)

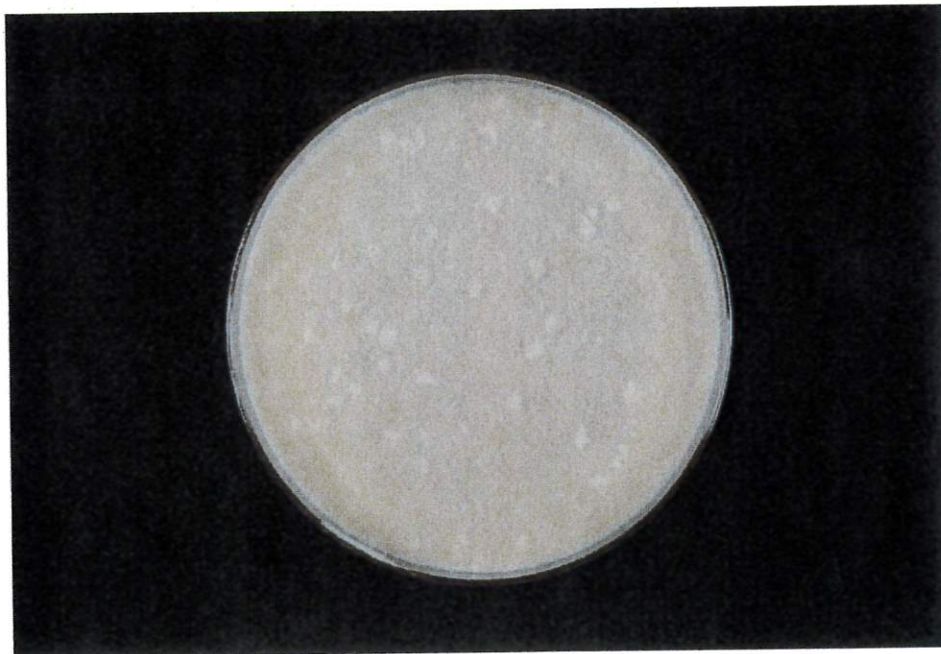


Photo. 37: *Aspergillus niger*, Control (1), After 1 minute
(0.1 mL of the test solution)



Photo. 38: *Aspergillus niger*, Test water, After 5 minutes
(0.1 mL of the test solution)

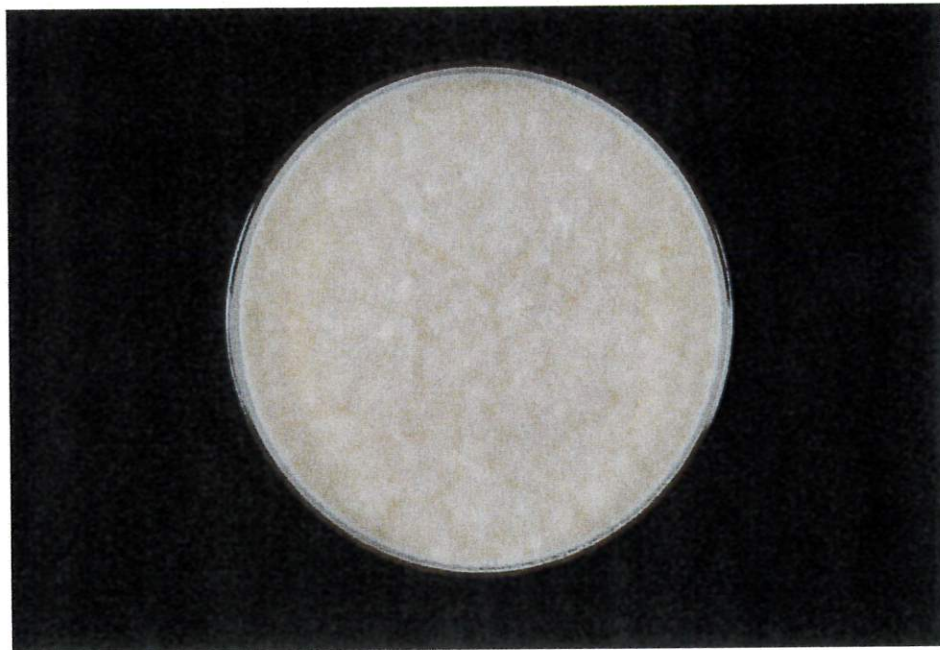


Photo. 39: *Aspergillus niger*, Control (1), After 5 minutes
(0.1 mL of the test solution)

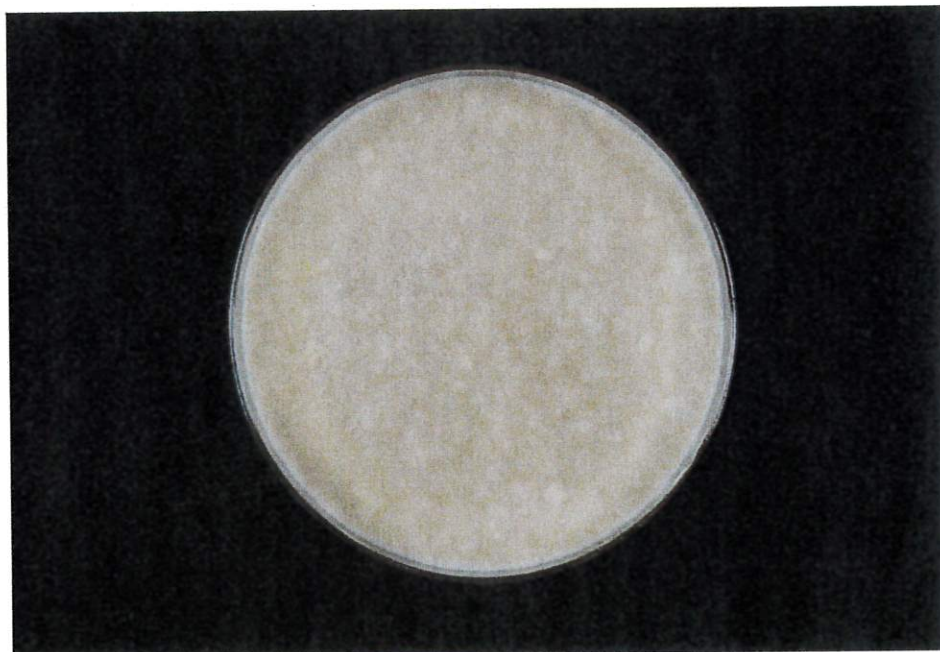


Photo. 40: *Aspergillus niger*, Control (2), After 5 minutes
(0.1 mL of the test solution)

End of Report