Biomedical Laboratory Sciences

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BLS ACADEMY
BIOMEDICAL LABORATORY SCIENCE

Clinical Case

BioTriCK

Public Health (Water Analysis)

Polytechnic of Coimbra

Introduction

Sample of water from a private well intended for human consumption

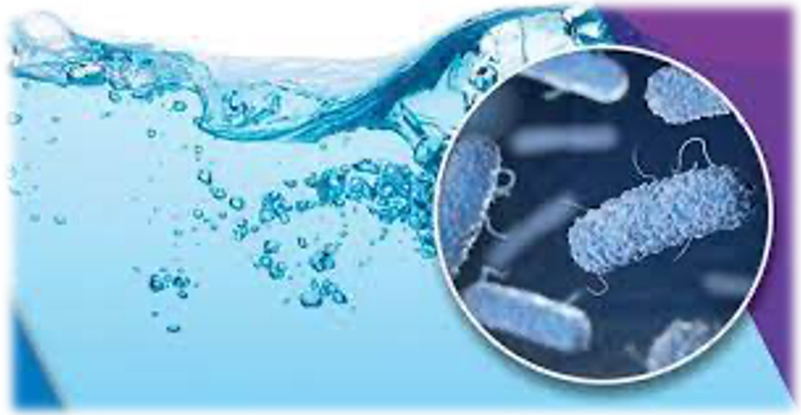
→ Microbiological study of water to evaluate if it is suitable for consumption

Decree Law n° 152/2017 → states that water for human consumption is all water in its original state or after treatment that is intended for drinking, cooking, food preparation, personal hygiene or other domestic functions

Methods and results

Microbiological parameters for drinking water

- Research of viable microorganisms at 36°C and 22°C
- Coliform bacteria research
- *Escherichia coli* research
- Research of fecal Enterococci
- *Clostridium perfringens* research



Methods and results

Research of viable microorganisms at 36°C and 22°C

Embedding method

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1ml sample + melted culture medium

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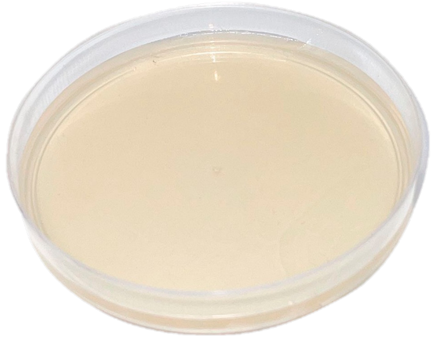
Homogenize

Used medium Plate Count Agar (PCA)



Incubation at (36±2)°C for 48 hours and at (22±2)°C for 72 hours under aerobiosis

Methods and results

Research of fecal Enterococci, Coliform bacteria, *Escherichia coli and Clostridium perfringens*

Membrane filtration method → Filtration of 100 mL of sample to the membrane that is subsequently placed in the culture medium

Methods and results

Research Coliform bacteria and *Escherichia coli*

Used medium Lauryl Sulphate Agar (LSA) for the research of *Escherichia coli* and coliform bacteria

Coliform bacteria - Incubation at (36±2)°C for 24 hours ;

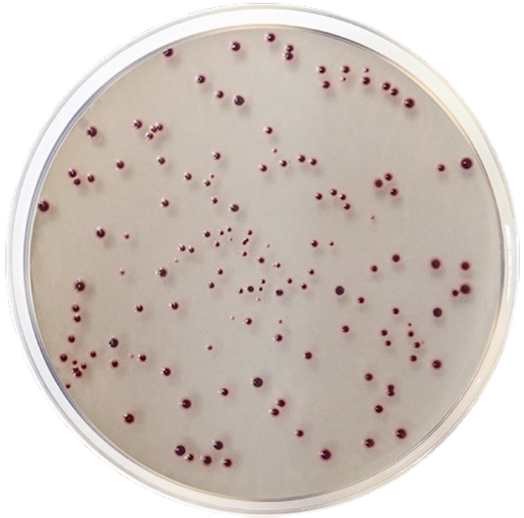
Escherichia coli – incubation at (44±0,5)°C for 24 hours;

Under aerobiosis

Methods and results

Research of fecal Enterococci

Used medium Slanetz and Bartley (SB) for the research of fecal Enterococci



Incubation at (36±2)°C for 48 hours;

Under aerobiosis

Methods and results

Research of *Clostridium perfringens*

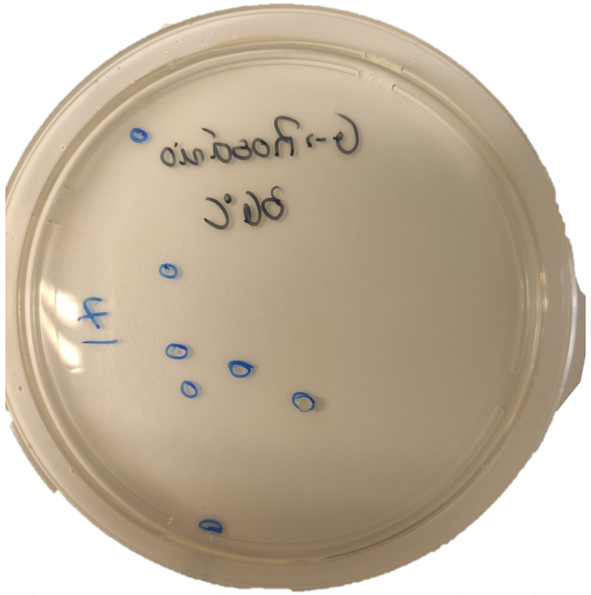
Used medium Tryptose-Sulfite-Cycloserine (TSC) for the research of *Clostridium perfringens*

Incubation at (44±0,5)°C for 24 hours;

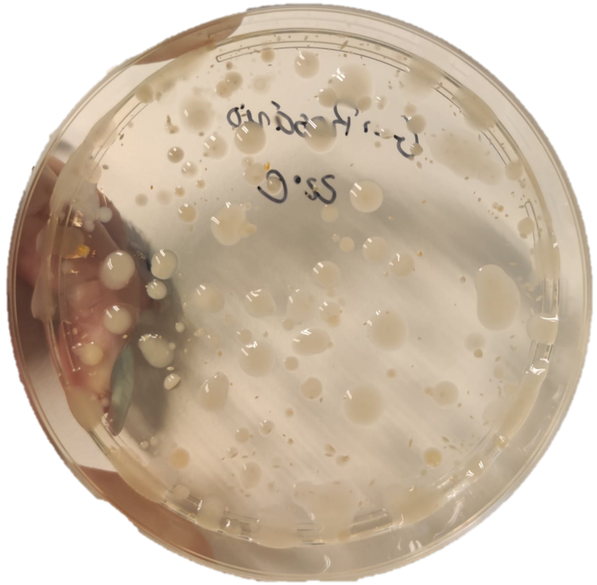
Under anaerobiosis

Methods and results

RESULTS:



PCA medium incubated at 36°C



PCA medium incubated at 22°C

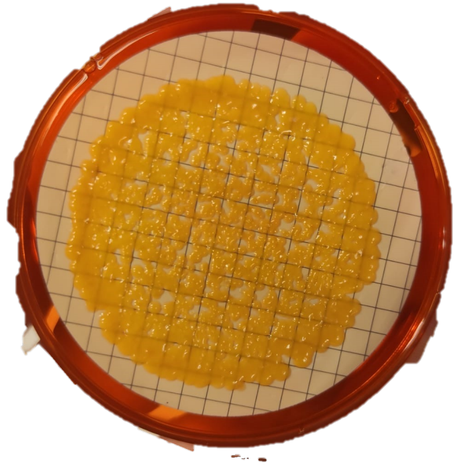
Presence of microorganisms on both plates

7 colonies on the plate incubated at 36°C

More than 300 colonies on the plate incubated at 22°C

Methods and results

RESULTS:



LSA medium for coliform bacteria research - a number of colonies greater than 80 was identified, with yellow coloration

5 colonies spiked to medium Nutrient Agar (NA) and incubated at 36°C for 24 hours

Oxidase proof

NEGATIVE

Sample contaminated by coliform bacteria

Methods and results

RESULTS:

LSA medium for *Escherichia coli* research - 2 yellow colonies and 3 orange colonies

1 yellow colony and 1 orange colony were spiked to the culture medium Nutrient Agar (NA) and incubated at 36°C for 24 hours

Colonies spiked to DEV-fluorocult medium and incubated at 44°C for 24 hours in aerobiosis.

Yellow colony
POSITIVE

Escherichia coli

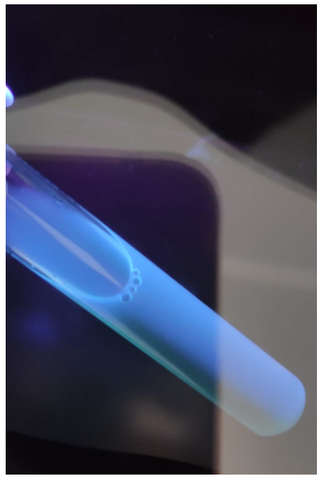
Orange colony
NEGATIVE

Other coliform bacteria

Methods and results

To confirm that the yellow colony is indeed an Escherichia coli, it was performed:

Fluorescence test

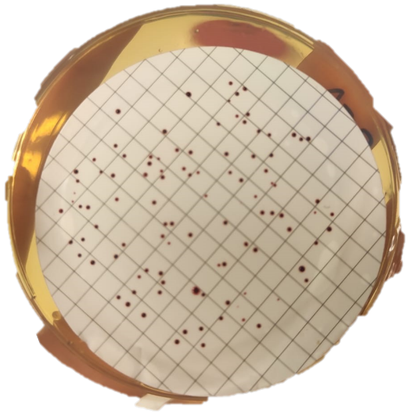


POSITIVE → ***Escherichia coli*** ← POSITIVE

Indole test

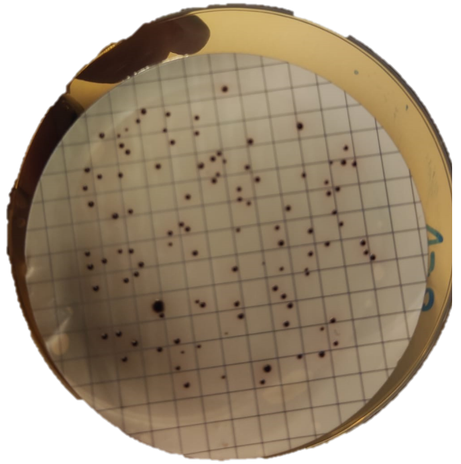
Methods and results

RESULTS:



Slanetz and Bartley (SB) for the research of fecal Enterococci - more than 80 brownish pink colonies

For confirmation, the membrane was transferred to the medium Bile Esculin Agar (BEA) and incubated at 44°C for 2 hours, in aerobiosis.



Blackening of culture medium

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POSITIVE

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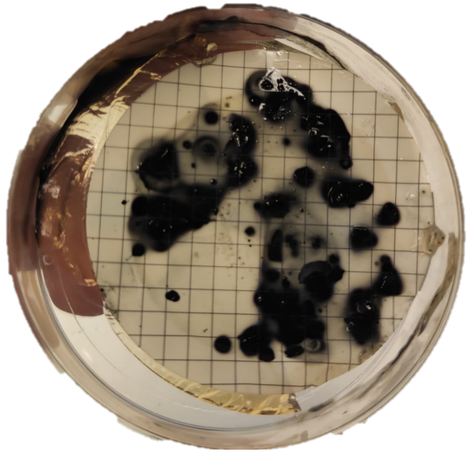
Fecal enterococci

Methods and results

R E S U L T S:



Medium Tryptose-Sulfite-Cycloserine (TSC) for the research of *Clostridium perfringens* - typical black colonies

For confirmation, an acid phosphatase test was performed

POSITIVE

↓

Clostridium perfringens

Discussion

Water intended for human consumption contaminated with viable microorganisms at 22°C and 36°C, coliform bacteria, Escherichia coli, fecal enterococci and Clostridium perfringens.

TREATMENT

- Water contaminated with these microorganisms can and should be treated using chemical (chlorine) and physical (ultraviolet) disinfection processes.

- The problem arises when these waters are also contaminated by clostridium perfringens, since it has the ability to produce very resistant spores, being little sensitive to common disinfection processes. In these cases, the most effective treatments are ultrafiltration and ozonation.

Conclusion

The analyzed water is unfit for consumption

It should not be used for domestic purposes and a specific and directed treatment should be carried out in order to improve the quality of the water.

Bibliography

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