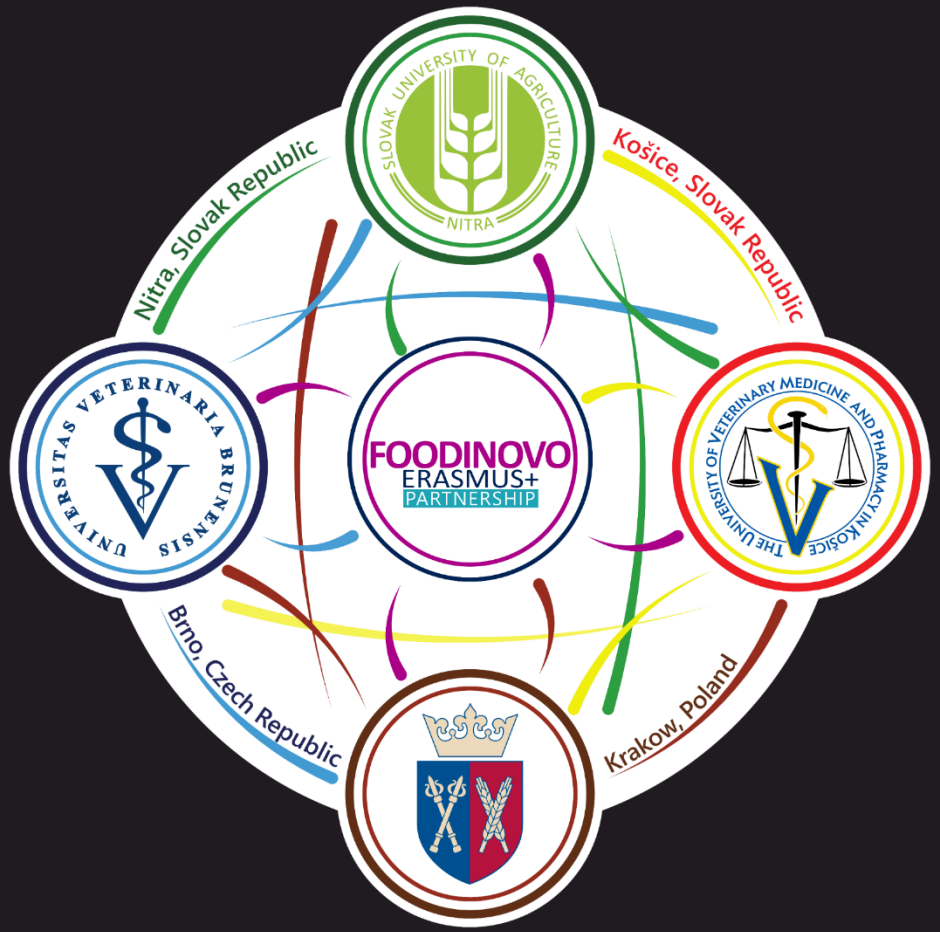
# Microbial quality of drinking water in laboratory

Practical part

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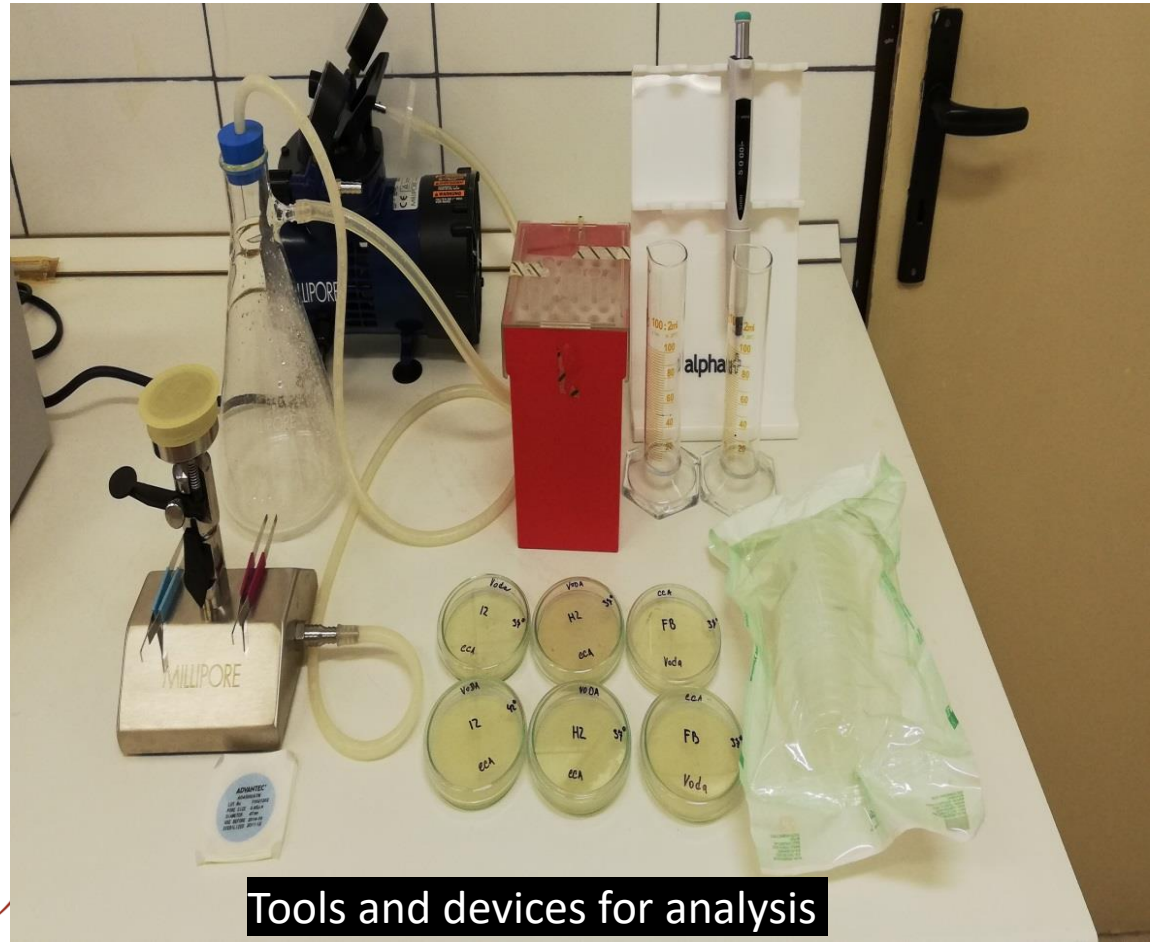
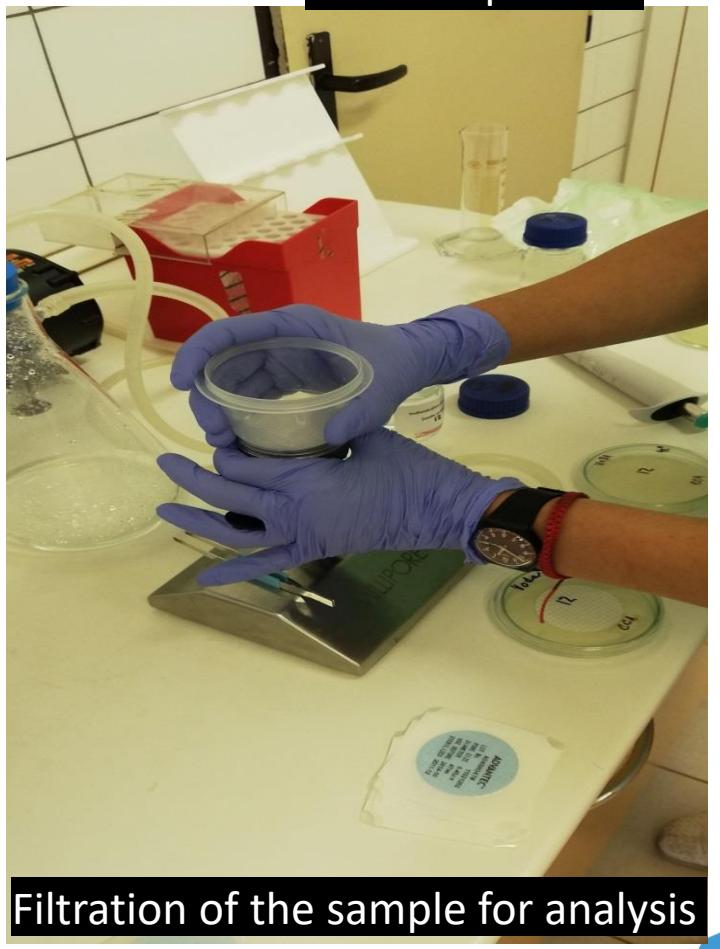
# Microbiological analysis of drinking water




Tools and devices for analysis

filtration process

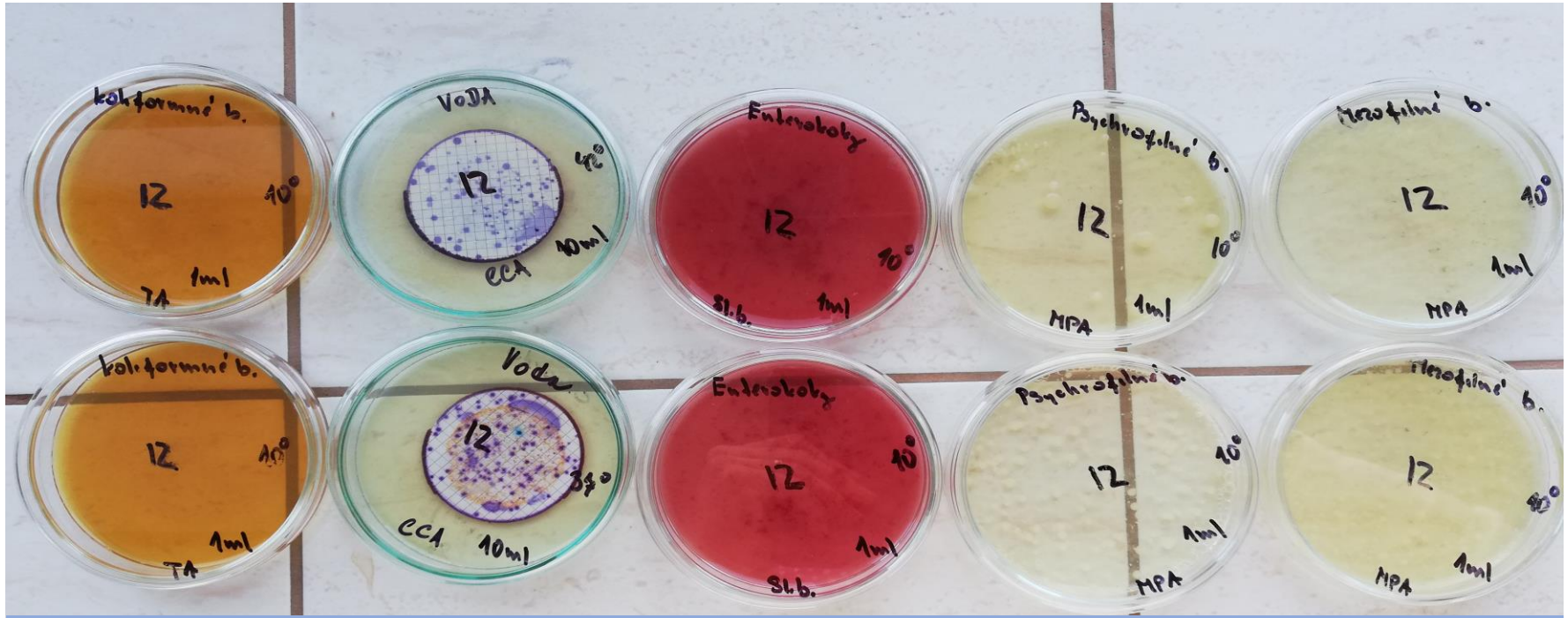


Filtration of the sample for analysis

# Determination of bacteria in clear water

## Plate method

- in the case of pure drinking water, inoculum 10/100/250ml directly from the undiluted

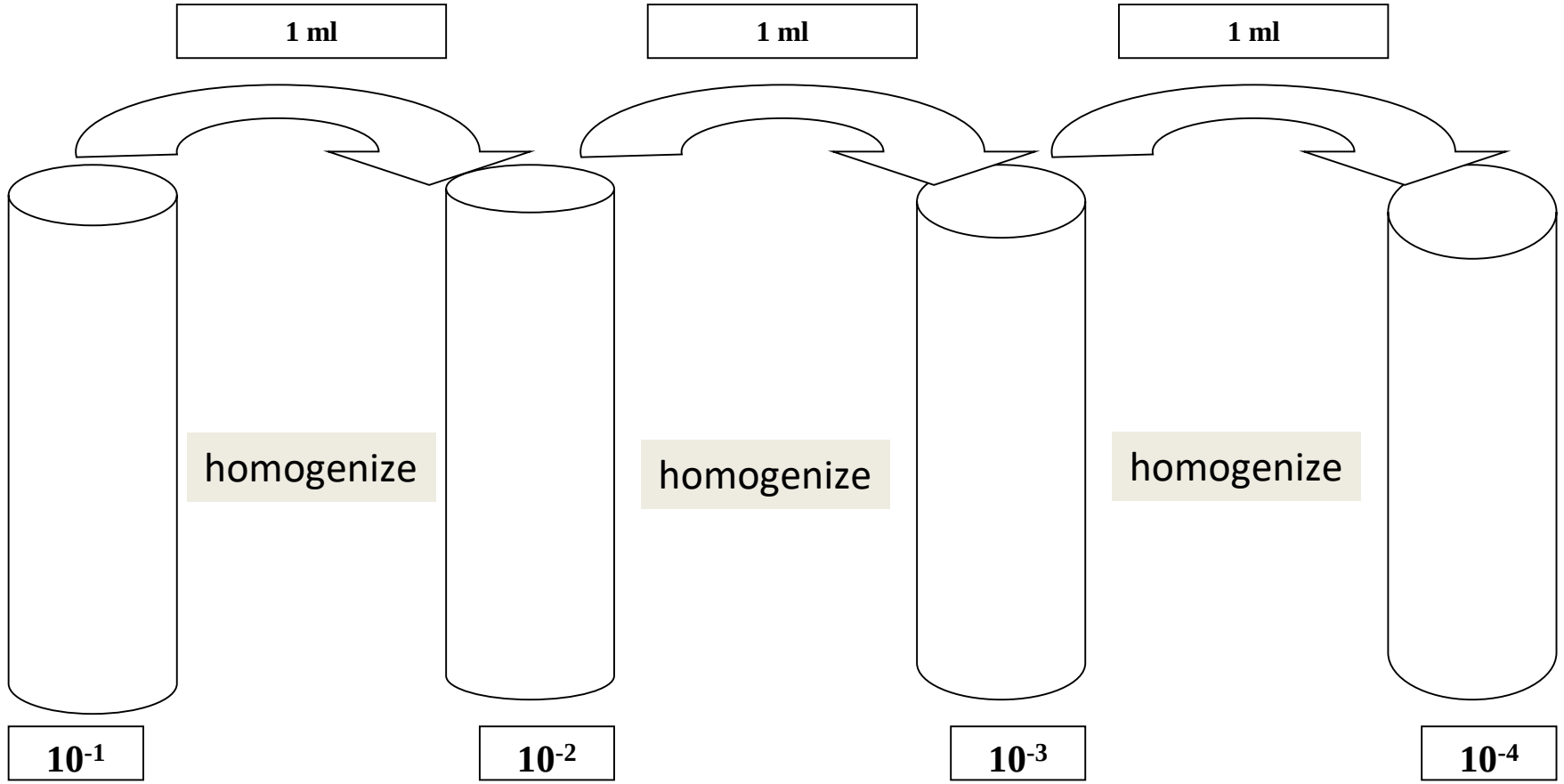



**If the water is significantly polluted, it must be diluted before determination**

# Determination of bacteria in waste water

**Inoculum - sample**

5 ml of waste water + 45 ml of saline / sterile water or
10 ml samples + 90 ml physiol. solution / sterile water
**The basic dilution is 1:10**

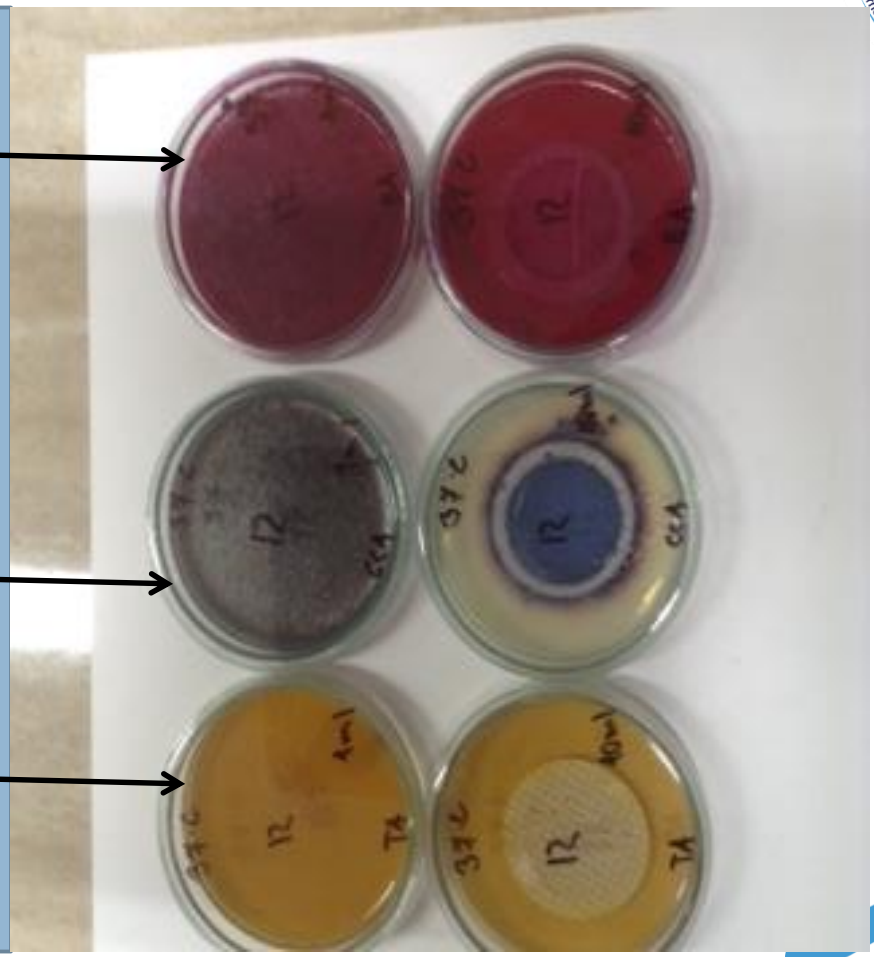


# Selective culture media for the determination of Coliform bacteria and *E. coli*

1. Endose agar with lactose (EA)

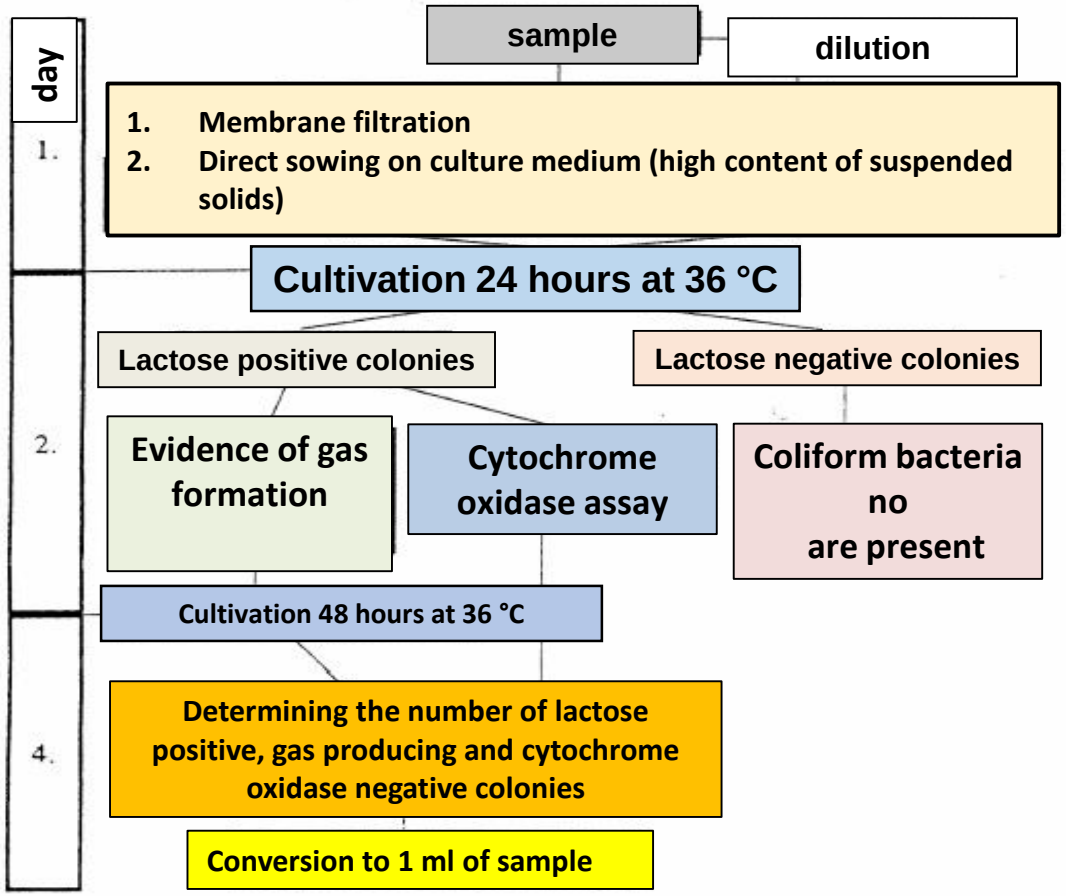
2. Chromogenic coliform agar (CCA)
   - lactose-free with sorbitol

3. Tergitol Lactose TTC Agar (TA)

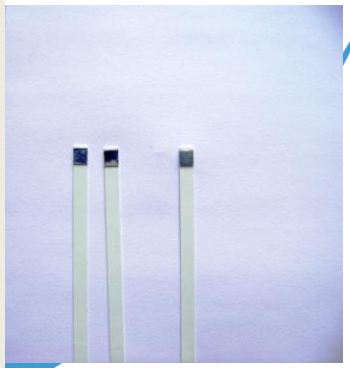


# Scheme for determination of coliform and presumptive *E.coli* in drinking water

Production of the enzyme β-D-galactosidase (lactase), which breaks down lactose into simple carbohydrates (D-glucose and D-galactose) - yellow color caused by acid formation by fermentation




**The positive OXI test has a dark blue color within 30 seconds. It also turns blue within 2 minutes - delayed positive reaction. A gray or greenish color after two minutes indicates a negative reaction.**



# Cytochrome oxidase test


**The oxidase test identifies the organisms that produce the enzyme cytochrome C oxidase (the last enzyme in the respiratory chain) - the transfer of electrons in the electron transport chain of aerobic bacteria to oxygen.**

**Principle: reaction of N, N-dimethyl-1,4-phenylenediamine and alpha-naphthol with the microbial enzyme cytochrome oxidase to give indophenol blue**

**Oxidase-positive: *Pseudomonas aeruginosa, Pasteurella multocida, Vibrio* sp., *Aeromonas* sp. or *Neisseria* sp.**

**Negative result: *E. coli, Klebsiella pneumoniae, Enterobacter cloacae, Serratia* sp. *or Acinetobacter* sp.**

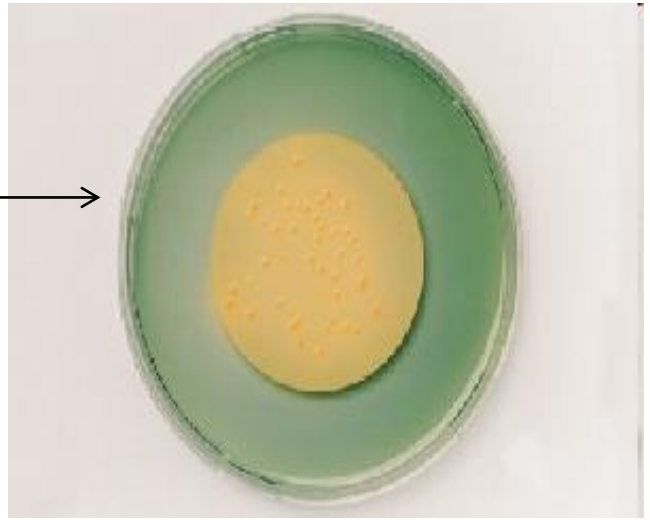
# Determination of coliform bacteria and *Escherichia coli* on membrane filters (mf) and Tergitol agar with TTC

Standard test

mf on TTC selective agar with tergitol

Cultivation 36 °C, 24 h

lactose positive, yellow court under filter



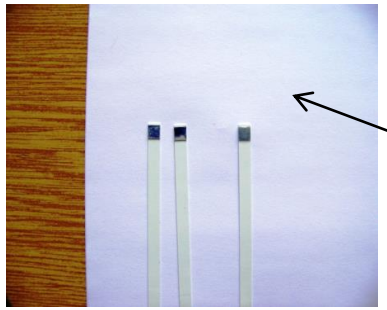
10 colonies, pure culture

non - selective tryptone - soy agar TSA

tryptophan agar

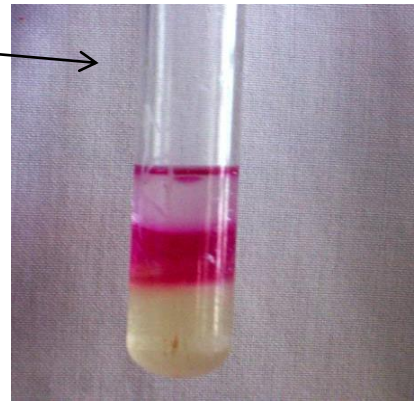
Cultivation 36 h

Cultivation 24 h



oxidase assay

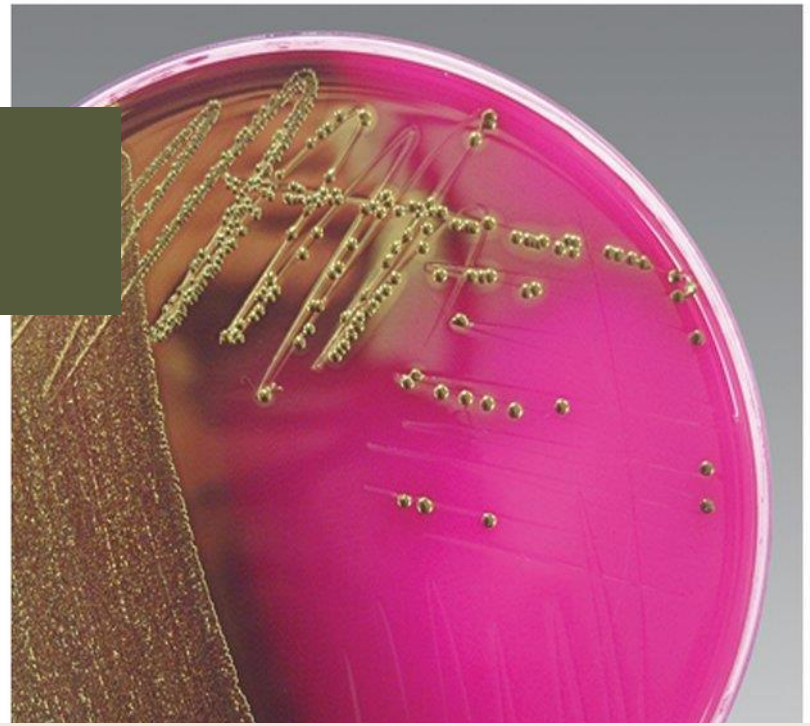
Indol test- Kovacs



Coliform bacteria, Oxidase negative

Escherichia coli , Oxidase negative Indol positive

# End's agar

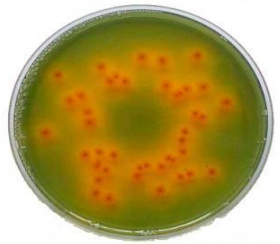


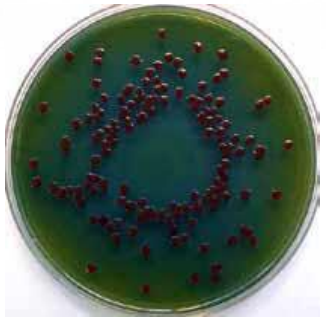
**Agar selectivity is created by a combination of sulfite and basic fuchsin, which suppress the growth of gram-positive microorganisms. Lactose-fermenting coliform bacteria form pinkish-red to deep red bulging colonies on Endo agar, in some cases with a metallic luster (*E. coli*). The medium around the colonies is also stained.**

**Microorganisms that do not ferment lactose are colorless, well observable against the pink background of the agar.**

# Evaluation of bacterial growth on Tergitol agar

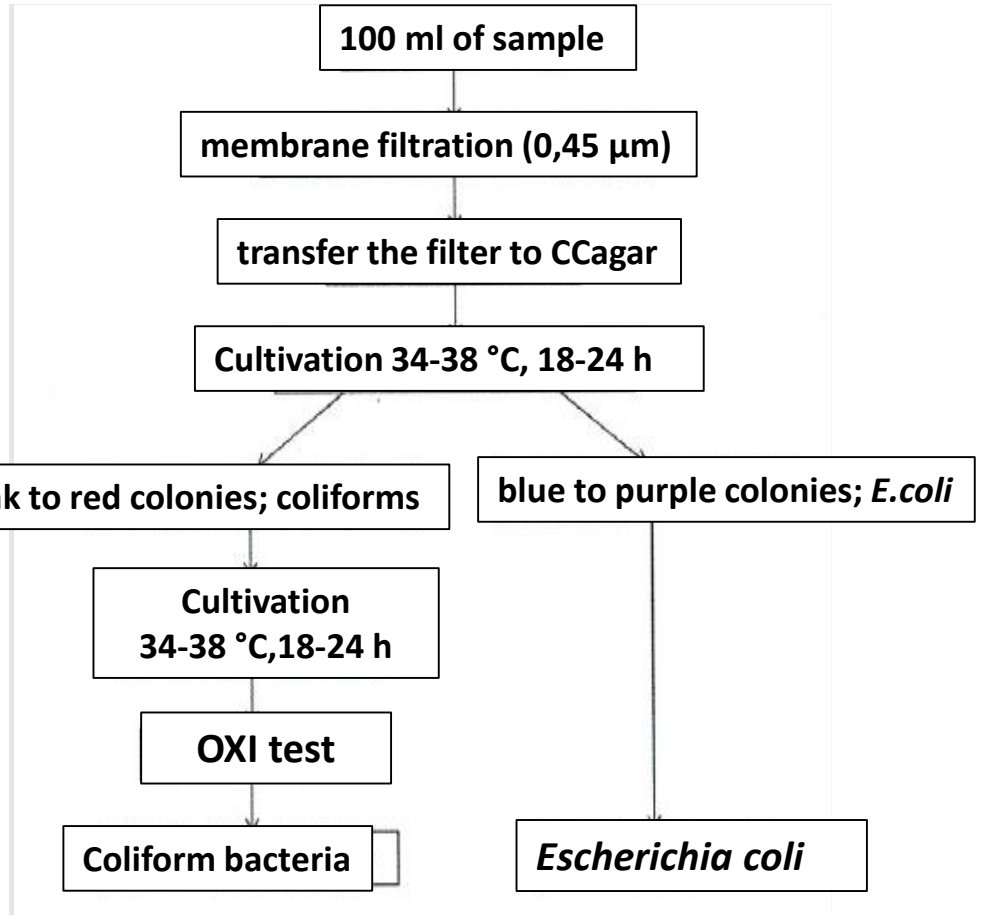
| *Escherichi coli* | yellow colonies in the yellow zone sometimes from the rusty red center |
|---|---|
| *Salmonella sp.* | red colonies with bluish zone |
| *Shigella sp.* | red colonies with bluish zone |
| *Proteus sp.* | red colonies with bluish zone |
| *Pseudomonas sp.* | red colonies with bluish zone |

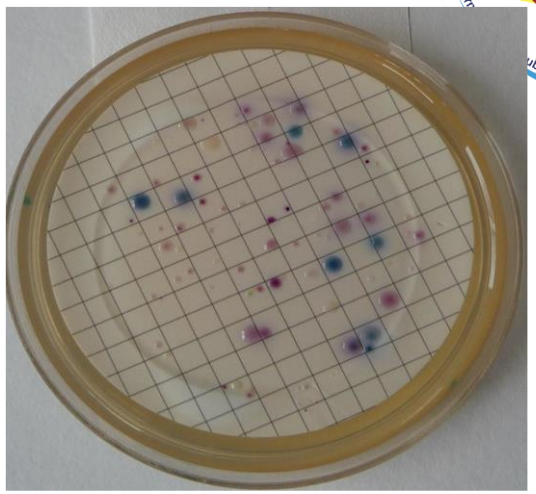


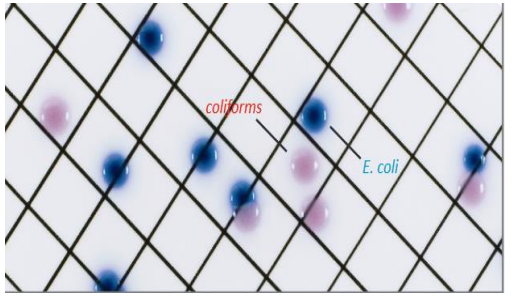


**Principle:**
**Tergitol agar inhibits the growth of G + bacteria and of the G- members of the genus Proteus.**
**The added TTC (3-phenyl tetrazolium chloride) is reduced to red formazan by the bacteria present except *E. coli* and *Enterobacter* sp.**

# Determination of coliform bacteria and *E.coli* on CCA



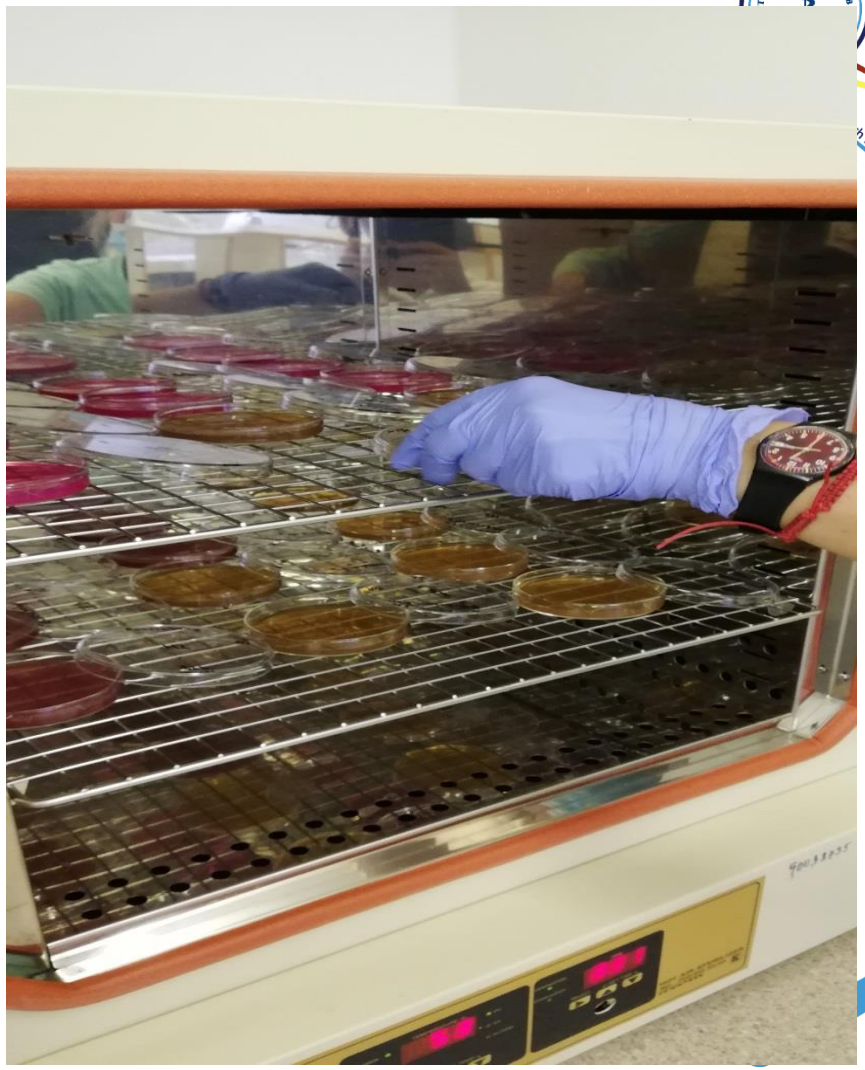


# Chromogenic coliform agar - CCA agar

B-glucuronidase-negative rare strains of *E. coli* are false negative on this medium (typically 0157 *E. coli*), but appear as coliform bacteria (i.e., pink colonies). If the research focuses on rare pathogenic strains such as *E. coli* O157: CHROMagarTM O157 should be used.

# Presumptive *Escherichia coli*

1. It meets all confirmatory tests as other coliform bacteria (lactase positive, oxidase negative, forms indole from tryptophan).
2. Positive evidence of β-D glucuronidase enzyme: hydrolyses MUG (4-methylumbelliferyl - β-D-glucuronide) as a blue-white fluorescence in a weakly alkaline environment under UV to 4-methylumbelliferone.



| Indicator | Drinking water | | | |
|---|---|---|---|---|
| | In system | BDW | In system | BDW |
| ***Escherichia coli*** | HLV | HLV | 0 CFU in 100 ml | 0 CFU in 250 ml |
| **Coliforms bacteria** | LV | LV | 0 CFU in 100 ml | |
| **Intenstinal enterococci** | HLV | HLV | 0 CFU in 100 ml | 0 CFU in 250 ml |
| **Cultivable at 22 °C/ Psychrophilic bacteria** | LV | LV | 200 CFU in 1 ml | |
| **Cultivable at 36 °C/ Mesophilic bacteria** | LV | LV | 50 CFU in 1 ml | |
| **Living organisms** | LV | - | 10 in ml (without desinfection) 0 in 1 ml (with desinfection) | |
| **Dead organisms** | LV | - | 30 v 1ml | |
| **Iron and manganese bacteria** | LV | - | 10 % field cover | |
| **Abioseston** | LV | - | 10 % field cover | |
| ***Clostridium perfringens with spores*** | IV | - | 0 CFU in 100 ml - only drinking water treated from surface water or groundwater | |
| **Microscopic fungi** | LV | - | 0 individuals in 1ml | |
| ***Filamentous bacteria (except iron and manganese)*** | LV | | 0 individuals in 1ml | |

**BDW - bottled drinking water; CFU- Colony Forming Units**
**Limit value (LV) - the value of the indicator, by exceeding which the drinking water loses satisfactory quality in the indicator, the value of which was exceeded.**
**Highest limit value (HLV) - the value of the water quality indicator with a threshold effect, the exceeding of which excludes the use of water for the intended purpose.**
**Indication value (IV) - the value of a non-specific or group water quality indicator used to assess the need for more detailed water quality tests.**

# Drinking water quality indicators by Government Regulation no. 91/2023 valid in Slovakia

This work was co-funded by the Erasmus+ Programme of the European Union

Innovation of the structure and content of study programs profiling food study fields with a view to digitizing teaching

FOODINOVO | 2020-1-SK01-KA203-078333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