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MICROBIOLOGICAL ANALYSIS OF WATERS

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Abstract. The objective of the bacteriological analysis of a water is not to make an inventory of all the species present, but to search for those which are likely to be pathogenic or, what is often easier, those which the companions and who are in greater numbers often present in the intestine of mammals and are by their presence indicative of fecal contamination and therefore diseases associated with fecal contamination. In this article, we will see how to perform a bacteriological analysis of water. An analysis that begins with the sampling procedure that must implement methods to ensure the absence of contamination of the sample and bacterial survival (storage conditions). The following are general methods of bacteriological examination of laboratory water; Finally, a selection of applied techniques is presented for the detection of pollution indicator bacteria and treatment efficacy (*Aerobic revivable bacteria*, total Coliforms, thermotolerant Coliforms, Enterococci, Sulphito-reducing Anaerobes), then specific bacteria, strict pathogens or opportunistic pathogens. It can be noted that the absence of faecal contamination does not suggest the absence of any potentially pathogenic species (eg.Salmonella, Staphylococcus, Pseudomonas).

Keywords: Bacteriological analysis of water, Membrane filtration, Indicative of fecal contamination, Pathogenic and Opportunistic Bacteria

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МИКРОБИОЛОГИЧЕСКИЙ АНАЛИЗ ВОДЫ

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Аннотация. Цель бактериологического анализа воды состоит не в том, чтобы провести инвентаризацию всех присутствующих видов, а в том, чтобы найти те, которые

могут быть патогенными, или те, которые часто встречаются у спутников и в больших количествах. Бактерии присутствуют в кишечнике млекопитающих и своим присутствием указывают на фекальное загрязнение и, следовательно, заболевания, связанные с фекальным загрязнением. В этой статье мы увидим, как выполнить бактериологический анализ воды. Анализ, который начинается с процедуры отбора проб, которая должна реализовывать методы, обеспечивающие отсутствие загрязнения образца и выживания бактерий (условия хранения). Ниже приведены общие методы бактериологического исследования лабораторной воды. Наконец, представлен набор применяемых методов для обнаружения бактерий-индикаторов загрязнения и эффективности восстановления (аэробные бактерии, общие колиформы, термотолерантные колиформы, энтерококки, сульфитредуцирующие анаэробы), затем специфические бактерии, строгие патогены или условно-патогенные микроорганизмы. Можно отметить, что отсутствие фекального загрязнения не предполагает отсутствия каких-либо из потенциально патогенных видов (например, Salmonella, Staphylococcus, Pseudomonas).

Ключевые слова: бактериологический анализ воды, мембранная фильтрация, индикация фекального загрязнения, патогенные и оппортунистические бактерии.

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I – DISEASES WITH WATER TRANSMISSION OF BACTERIAL ORIGIN

I-1 – Typhoid and paratyphoid fever

They are true septicemia due to Salmonella: *Salmonella typhi* and *S. paratyphi* A, B, C. They are characterized by a fever accompanied by an extreme abatement and can have serious complications, sometimes mortal: intestinal hemorrhages. Contamination occurs through the digestive tract from water contaminated with faeces, spoiled food or dirty hands.

I-2 – Cholera: causal agent?

Short incubation disease ranging from a few hours to 5 days. It is accompanied by vomiting and epigastric pain with anuria and muscle cramps. Its evolution is fatal in the absence of rehydration and antibiotic therapy. The mechanism of action is due to a 103 amino acid toxin that binds to the duodenum cells responsible for the absorption of water from the intestinal lumen to the inner environment and reverses the mechanism, leading to a loss of water 8 to 10 l/d.

1-3 – Acute gastroenteritis

Disease caused by Escherichia coli strains called enterohemorrhagic EHEC. These cause bloody diarrhea and produce a powerful toxin. Although very numerous, these bacteria are hardly pathogenic: 5 to 6 % of strains only in children. Only in very rare cases do they enter the blood, causing sepsis or urinary tract infections. Short incubation disease ranging from a few hours to 5 days. It is accompanied by vomiting and epigastric pain with anuria and muscle cramps. Its evolution is fatal in the absence of rehydration and antibiotic therapy.

II – BACTERIA RESEARCHED IN WATER

II-1 – Total Aerobic Mesophilic Flora

Definition

Total aerobic mesophilic flora is a health indicator that can be used to evaluate the number of CFUs (Colony Forming Units) present in a product or on a surface. This count is at 30 °C which allows to count three major types of flora: Thermophilic flora, optimal growth temperature at 45 °C; Mesophilic flora, optimal growth temperature between 20 °C and 40 °C. The psychrophilic flora, optimum temperature of growth at 20 °C. As this is an ordinary medium, most microorganisms can grow except those that are demanding and strict anaerobic microorganisms. It is therefore better to talk about Mesophile Aerobic Flora at 30 °C than "total flora". The unit is the CFU (Colony Forming Unit) because an observable colony on the agar may come from an isolated microorganism, a spore or an association of microorganisms.

II-2 – The Coliforms

II-2-a – Total coliforms

Definition

Total Coliforms are Gram negative (BGN), aerobic or facultative anaerobic bacilli, non sporulating, oxidase negative, capable of multiplication in the presence of bile salts, and capable of fermenting lactose with the production of acid and gas in 24 to 48 hours at a temperature between 36 /–2 °C. These are bacteria used as an indicator of the microbiological quality of water. Either 10 coliforms per mL and more, announces a contamination of drinking water.

Although they do not belong to the coliform group, Aeromonas bacteria have the β-galactosidase enzyme and can ferment lactose, leading to false positive results for total coliforms. Aeromonas species are widespread in the environment and exist among others in lakes, rivers, seas, sewage effluent and drinking water.

Table 1 – Some coliforms of the Enterobacteriaceae family

Coliform group	*Ortho-nitrophenyl-beta-D-galactoside*	Fecal origin	No fecal origin
Budvicia	+	–	+
Citrobacter	+	+	+
Enterobacter	+	+	+
Erwinia	+	–	+
Escherichia	+	+	–
Klebsiella	+	+	+
Leclercia	+	–	+
Pantoea	+	–	+
Serratia	+	–	+

II-2-b – Fecal Coliforms

Definition

Fecal coliforms, or thermo-tolerant coliforms, are a subset of total coliforms capable of fermenting lactose at a temperature of 44.5 °C. The species most

frequently associated with this bacterial group is *Escherichia coli* (*E. coli*) and, to a lesser extent, certain species of the genera Citrobacter, Enterobacter and Klebsiella. However*, E. coli* bacteria account for 80 to 90 % of the detected thermo-tolerant coliforms. The interest in detecting these coliforms as indicator organisms lies in the fact that their survival in the environment is generally equivalent to that of pathogenic bacteria and that their density is generally proportional to the degree of pollution produced by the bacteria feces. Moreover, since fecal coliforms do not usually proliferate in a distribution network, they are useful for verifying its water-tightness, making it possible to detect fecal contamination resulting for example from infiltration of polluted water into the pipes. They are also good indicators of the effectiveness of water treatment, but since they are less numerous than total coliforms, they are preferable for this function.

II-3 – Fecal Streptococci (Intestinal Enterococci)

Definition

It is Gram Positive Cocci (CGP) spherical or ovoid, in chains more or less long, non sporulating, aero-anaerobic facultative, possessing neither catalase nor oxidase. The persistence of Enterococci in various water types may be greater than that of other indicator organisms, in particular because of their known resistance to disinfectants, which makes them preferred indicators for assessing the efficacy of the treatment. water. In addition, their high resistance to drying makes Enterococci indicators for control during repairs to the distribution network requiring drying. Moreover, since there is generally no growth of Enterococci in a distribution network, their detection usually indicates recent faecal pollution. In this context, the role of enterococci as an indicator of faecal contamination in aquifers (groundwater) has recently been recognized, as studies in the United States have demonstrated their usefulness in detecting faecal contamination of groundwater. groundwater. This interest in Enterococci is explained by the fact that, compared with coliforms (including Escherichia coli), they are more resistant to harsh environmental conditions and persist longer in water; such conditions are typical of groundwater where the temperature is generally colder and nutrient poor.

II-4 – Sulphito-reducing Anaerobes

Definition

Spores of anaerobic sulfite-reducing microorganisms (clostridium) are widely distributed in the environment. They are present in human and animal faeces, as well as in wastewater and soil. Unlike *Escherichia coli* and other conforming organisms, spores survive in water for a long time because they are more resistant than vegetative forms to the action of chemical and physical factors. They can thus provide indications on distant or intermittent pollution. They can even be resistant to chlorination in the proportions usually used for water treatment, and are thus useful for the purposes of controls. Their presence in the absence of fecal germs in the water can be interpreted as a lack of protection of the water table against the presence of a foreign bacterial flora. Spores are a good indicator for these microorganisms. In addition, these forms resist chlorination. This explains that for this particular parameter, it is not the bacteria themselves but their spores that are sought.

II-5 – *Vibrio cholera*

Definition

Vibrios are gram negative, curved, aerobic-anaerobic bacilli that can be moved by a single polar eyelash. Vibrio cholerae, responsible for cholera, was discovered in 1854 by PACINI in Florence and cultivated in 1883 by R. KOCH in Cairo. Vibrio cholerae is found in the stools of the sick and some subjects (healthy carriers). It survives in polluted waters as well as on contaminated objects.

II-6 – Salmonella

Definition

Salmonellae are enterobacteria whose essential characteristics are not to ferment lactose and not to produce urease. Salmonella belongs to the Enterobacteriaceae family. These are bacilli Gram negative, moving for most (ciliature peritrichous), but some are stationary, optional aero-anaerobic, oxidase-, nitrate reductase + fermentative glucose, lactose – H2S + (or-), urease- , lysine decarboxylase +, using the way of the mixed acid, indole, lacking beta-galactosidase, highly contagious, responsible for gastroenteritis, food poisoning and typhoid and paratyphoid fevers (S. typhi and S. paratyphi). They cause diseases such as typhoid fever, paratyphoid fever and salmonellosis in humans, one of the main causes of collective food poisoning (TIAC). The main mode of contamination in humans is ingestion in humans. from water (especially S. typhi), food (eg dairy products, eggs, meat) or carrier pets (turtles).

II-7 – *Pseudomonas aeruginosa*

Definition

Pseudomonas are bacteria GRAM (–), bacilli right and isolated or diplobacillus, oxidase (+), Glucose (–), Lactose (–), no production of H2S (–), Indole (–), strict aerobic, oxidase positive, mobile, naturally resistant to very many antibiotics. Saprophytes, they are found mainly in water. They can contaminate intravenous fluids, antiseptic solutions, liquid drug preparations. The main species, *Pseudomonas aeruginosa*, has the following characteristics:

- Proteolytic,
- *P. aeruginosa* secretes a number of pigments: among others pyocyanin (blue-green), pyoverdine (yellow-green fluorescent).
- Production of a necrotizing exotoxin by some strains.
- *Pseudomonas aeruginosa* expresses its pathogenic potential when introduced into areas with diminished immune defenses. Major opportunist, he is thus responsible:
- "Blue pus" suppurations of wounds and burns;
- Septicemia in the burned;
- Superinfection of the bronchi in cystic fibrosis, thanks to the production of elastase.

II-8 – *Staphylococcus aureus*

Definition

The bacteria of the genus Staphylococcus are Gram-positive hulls (Cocci), grouped into clusters in the form of bunches of grapes, immobile, non-sporulating, catalase positive and oxidase negative. Among the 27 species of the genus currently listed, there is the species

S. aureus which is a commensal of the skin and mucous membranes of man and animals (rhino-pharynx, intestine). It is found on the nasal mucosa of about one-third of normal subjects. Eliminated in the external environment, this bacterium can survive for a long time in the environment. Pyogenic germ by excellence, *S. aureus* is the microbe of suppuration. Staphylococci 30/122 Bacteriology – Bacteriology Service 2002–2003.

Some strains also act by release of one or more toxins (food poisoning, toxic shock syndrome, impetigo).

III – Sampling equipment

III-1 – Choice and sterilization of containers

The container used must once clog, a total protection against any contamination and must not yield toxic substances for bacteria. One can use either 500 and 1000 mL glass vials or single-use plastic vials sterilized by the manufacturer. Before use, the glass vials and stoppers are carefully washed, rinsed, dried and then individually wrapped in a piece of paper and then filter sterilized either by autoclaving (120 °C) for 15 min or at the Pasteur oven (170 °C) during 1h. It is desirable to arrange each bottle in a case adapted to its size, to ensure its protection during transport and prevent tearing of the filter paper envelope.In the case of sampling of chlorinated or ozonated water, the disinfectant may, during transport, continue to exert its action on the bacteria present, and it is necessary to destroy it. Add to the sampling bottle before sterilization of sodium thiosulfate at a rate of 17.5 mg/l (approximately 8mg per 500 mL bottle).

III-2 – General methods of sampling

III-2-1 – Sampling at the tap

In this case, the manipulation is carried out under the best conditions of sterilization. Before proceeding with the actual sampling:

- Remove the baffles and rubber hoses adapted to the selected faucet, remove if necessary limestone concretions, which may have been deposited there.
- Wash hands and forearms very thoroughly, rinse with alcohol, allow to dry.
- Flame the faucet for at least 1 min, using, for example, a portable butane gas torch.
- Open the tap and let it run for 3 to 5 minutes before taking the sample. During this wait, and during the sampling, it is useful for an assistant to keep the soldering lamp on, a little above the tap; the operator, in his manipulations, can use it as a Bunsen burner in the laboratory.
- After clearing the sterile packaging, take the flask with your left hand, bring it close to the free fingers of your right hand, remove with them cotton corking the neck.

- Quickly flaring the edge of the neck; fill the bottle almost completely, flaring quickly again the edge of the neck and put the cap on.
- Once the sample has been collected, write on the label the information needed to identify the sample. Replace the bottle in its paper envelope, preferably protecting the cap and the neck. On an attached sheet, write down all the information you need to interpret the analysis. Introduce everything, possibly, into the metal case.

III-2-2 – Griffon sampling from sources

It is advisable to isolate first the point of emergence of the water, and to prepare a location of capture, either by pushing in the griffon a pipe which will channel the water and facilitate the sampling is, if the water goes out without gushing out of the ground, by arranging a channel.Anyway, these manipulations should be done immediately before the sampling, but at least 24 hours in advance.

III-2-3 – Sampling in a well using a plunger

- Use a sterile plunger wrapped in a protective paper and a rope, the length of which is adapted to the depth of the well, terminated by a carabiner (= metal loop that a spring blade or an articulated lug keeps closed constituting a safety clip).
- Place the plunger on the ground, tear the upper part of the paper so as to release on the one hand the packet of the cap, on the other hand the loop of the cord.
- Engage this loop in the carabiner of the rope, release with the left hand all the upper part of the apparatus and in particular the neck of the bottle.
- During these operations avoid putting the rope in contact with the ground.
- Remove the cotton closing the neck, lift the device, release it completely from the paper and slowly lower it into the well, avoiding touching the walls of it.
- If the sample is made about 50 cm from the surface, which is the most common case, stop shortly before the carabiner, connecting rope and rope, reaches this surface.
- When the bottle is full (no bubbles coming out of the plunger), reassemble the device, disengage the bottle by taking it by the base of the neck (the handle of the plunger being lowered to clear the opening), to empty some of water, possibly flame the edge of the neck, and seal it with the cap clear of its package.

III-2-4 – Sampling of seawater

The strategy and the sampling plan depend on the objectives and criteria set:

- The first: taking deep water.
- The messenger bottles are available in two positions, horizontal and vertical. The choice of one or the other must be made according to the knowledge of the environment and mainly of the currents; as well as conditions of the surface of the water to the right of the levies.
- The trigger is the messenger, the opening of the cap or the overturning of the bottle must be done when the cable is in the upright position. Obtaining the verticality of the device and therefore the insurance.
- The second corresponds to sampling in the nearshore strip.

- Investigations in this area require a boat but generally small (between 5 and 10 meters). The possibility of reagent storage is generally almost impossible and, depending on the state of the sea, the addition of these reagents is very delicate, even dangerous. In this case, the bottles will be equipped with reagents, and their filling should be done carefully so as not to overflow the sample and thus lose all or part of the reagent.

Bottles

In view of the parameters envisaged for the analyzes, it will be important to use specific packaging for the bottle. Material characteristics and type of closure can influence and modify the sample.

Contamination

Depending on the type of study you must be aware that all the means used can disturb the environment. Boats, gear and sampling equipment are sources of contamination. The use of disposable gloves is proving to be an essential reflex during the various sampling and sampling operations.

III-3 – Transport and conservation in the laboratory

The initial germ content of the water may be modified in the bottle after collection. That's why any analysis should be done as quickly as possible. If the transport time exceeds 1 hour and the outside temperature is above 10 ° C, the samples must be placed in a refrigerated chamber (cooler).The vials should be kept at 4 to 6 ° C in a suitable container. A mobile refrigerator connected to the vehicle battery is preferable to a portable cooler. The ideal is to have an insulated vehicle whose entire box is refrigerated. It is therefore accepted that the maximum delay between the sampling and the start of the analysis must not exceed 24 hours, the sample being kept below + 4 ° C and that it is preferable to shorten this period when the water is presumed to be highly polluted. After taking the test, it is recommended to place the remainder of the unused sample in the refrigerator. It may happen that the first bacteriological readings, 24 or 48 hours after seeding, give unexpected results, prompting to verify the analysis.

IV – Samples, Germs sought and Culture Media used in cultivation of Bacteria

Germs sought	Culture Media used in cultivation of Bacteria	Volume of water to analyze, ml
a – Mineral water, Spring and Drilling		
Total Aerobic Mesophilic Flora	TGEA Agar	1
Total coliforms and Fecal Coliforms	TTC Tergitol 7 Agar	250
Pseudomonas aeruginosa	Cetrimide Agar	250
Fecal Streptococci	Slanetz and Bartley Agar, BEA (Bile-Esculin-Azide)	250
Sulphito-reducing Anaerobes	*Liver Meat Agar*	50
Salmonella	*Rappaport Broth, SFB Medium*	500
Staphylococcus aureus	Chapman Agar	250
b – Well water		
Total coliforms and Fecal Coliforms	TTC Tergitol 7 Agar	100
Fecal Streptococci	Slanetz and Bartley Agar, BEA	100

Germs sought	Culture Media used in cultivation of Bacteria	Volume of water to analyze, ml
	(Bile-Esculin-Azide)	
Sulphito-reducing Anaerobes	*Liver Meat Agar*	20
Salmonella	*Rappaport Broth, SFB Medium*	500
c – Swimming pool water		
Total coliforms and Fecal Coliforms	TTC Tergitol 7 Agar	100
Pseudomonas aeruginosa	Cetrimide Agar	100
Fecal Streptococci	Slanetz and Bartley Agar, BEA (Bile-Esculin-Azide)	100
Sulphito-reducing Anaerobes	*Liver Meat Agar*	20
Staphylococcus aureus	Chapman Agar	100
d – Tap water		
Total coliforms and Fecal Coliforms	TTC Tergitol 7 Agar	100
Fecal Streptococci	Slanetz and Bartley Agar, BEA (Bile-Esculin-Azide)	100
Sulphito-reducing Anaerobes	*Liver Meat Agar*	20

Table 2 – ISO standards used in bacteriological analysis of waters

Parameter	Standard
Enumeration of revivable microorganisms at 22 °C, 30 °C and 37°C	ISO 6222:1999
Enumeration of total Coliforms	ISO 9308-1:2000
Enumeration of *E. coli*	ISO 9308-1:2000
Enumeration of intestinal Enterococci	ISO 7899-2:2000
Enumeration of Spores of Anaerobic Sulfite-Reducing Bacteria	NF T90-415 (85)
Research of *Staphylococcus aureus*	XP T90-412
Research of Salmonella	ISO 19250:2010
Research of *Pseudomonas aeruginosa*	ISO 16266:2006
Research of *Vibrio cholerae*	ISO/TS 21872-1:2007

Enumeration of revivable microorganisms at 22 °C and 37 °C Incorporation technique

- In the sterile area.
- Mix the water bottle to be analyzed and aseptically take 1 ml in duplicate in two empty Petri dishes, numbered and prepared.
- From the water to be analyzed, dilutions 10^{-1} and 10^{-2} are made (Scheme 1).
- Distribute the inoculum into the Petri dishes.
- Then complete with approximately 20 ml of TGEA (TRYPTONE GLUCOSE EXTRACT AGAR).
- Then make "8" shaped movements to allow the inoculum to mix with the agar.
- Let the Petri dishes solidify on the bench.
- Incubate the Petri dishes: The first series will be incubated at 22 °C for 72 hours and the second series will be incubated at 37 °C for 24 hours.

Reading and interpretation

Colonies of microorganisms appear in bulk in lenticular and distinct forms.

Hold the dishes containing less than 300 colonies at two successive dilutions. A box must contain at least 15 colonies. Then calculate the value of the number N, of revivable microorganisms at 22 °C apart and that of the N number of microorganisms revivable at 37°C apart, as a weighted average, using the following equation:

$$N = \frac{\Sigma c}{1,1 \times d'}$$

Σc: is the sum of the colonies counted on two boxes of successive dilutions retained.

d: is the dilution ratio corresponding to the first dilution.

Round the calculated results to two significant digits after the decimal point.

The final result of revivable microorganisms counted at 22 °C and 37 °C per ml of water is denoted by a number between 1.0 and 9.9 multiplied by 10^x where x is the appropriate power of 10.

Example

A count of microorganisms at 37 °C gave the following results:

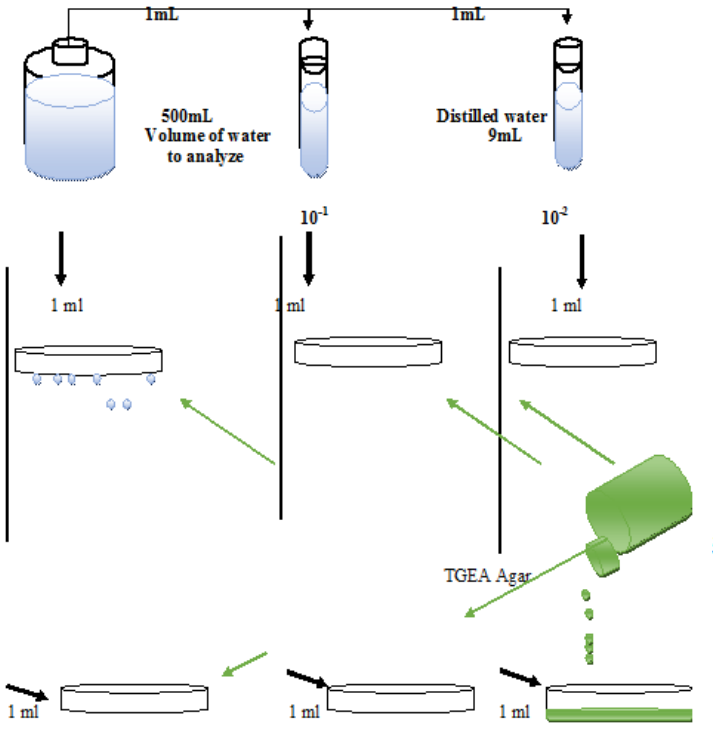
at the first dilution retained 10^{-2}: there are 158 colonies.

at the second dilution retained 10^{-3}: we have 14 colonies.

$$N = \frac{\Sigma c}{1,1 \times d} = \frac{148 + 24}{1,1 \times 10^{-2}} = \frac{172}{0,011} = 15636.$$

Round to two significant digits: 16000

1.6×10^4 microorganisms per mL of water at 22 °C or 37 °C.



Scheme 1 – Enumeration of Microorganisms Revivifiable at 22 °C and 37 °C

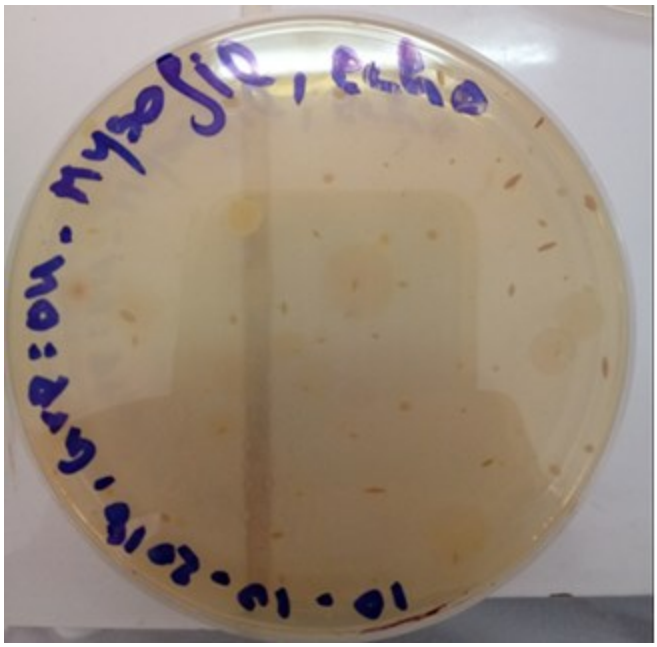
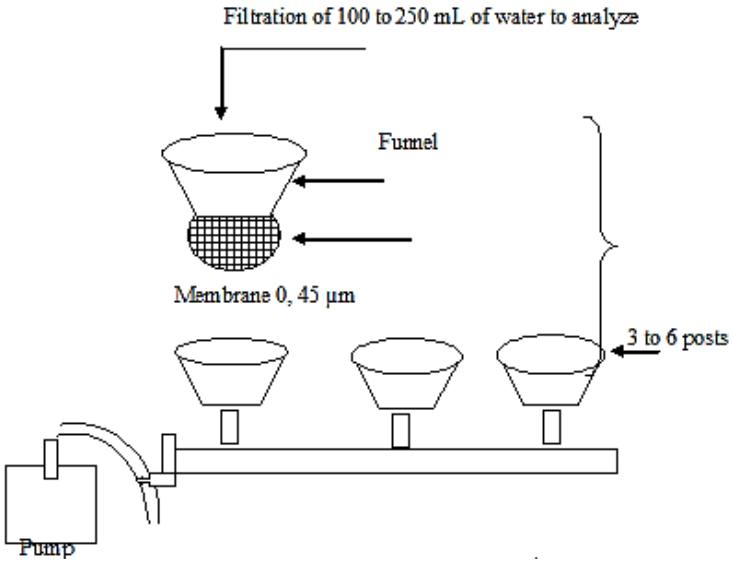


Figure 1 – Appearace of total on PCA

Enumeration of total and fecal Coliforms
Membrane filtration technique



Scheme 2 – Filtration system

The search for coliforms by membrane filtration is done according to the following steps:

- Sterilize the graduated funnel and the porous membrane with a bunsen burner.
- Cool them immediately afterwards with the water to be analyzed if it is available in sufficient quantity or with sterile distilled water.
- Aseptically install a 0.45µm porosity membrane between the porous membrane and the funnel using a sterile forceps.
- Then aseptically deposit 100 to 250 ml of water to be analyzed, depending on the type of water to be analyzed, in front of a bunsen burner.

- Then activate the vacuum pump to absorb water through the membrane.
- Remove the funnel and immediately and aseptically transfer the membrane using a sterile forceps onto the surface of a previously prepared TTC Tergitol agar plate.
- Incubate at 36 ± 2 °C for 21 ± 3 hours.

This box will be used to count coliform bacteria, followed by biochemical identification.

Reading and interpretation

Count the colonies after incubation in the form of small pigmented colonies of yellow-orange or yellow (positive lactose). Repeatly transplant 5 colonies on Triple Sugar Iron (TSI) agar for confirmation based on the oxidase test and indole production.

Oxidase test

- Deposit a characteristic colony on an oxidase disk.
- The positive reaction is immediate and translates into a dark purple turn.

Indole test

- Transfer each characteristic colony separately into a tube containing 3 ml of tryptophan broth or indole-free peptone water broth.
- Incubate at 44 ± 0.5 °C for 21 ± 3 hours (water bath).
- Look for indole production by adding 2 to 3 drops of Kovacs reagent.
- The presence of a red stain on the surface of the broth reflects the production of indole from the tryptophan present in the medium.

In conclusion

Germ	Feature	Oxidase test	Indole test
Coliform	yellow colony	–	(–) 44 °C
Escherichia coli	Orange-yellow colony	–	(+) 44 °C

- Count the colonies characteristics in each case.
- The results will be expressed in colony-forming unit CFU according to the type of water to be analyzed.

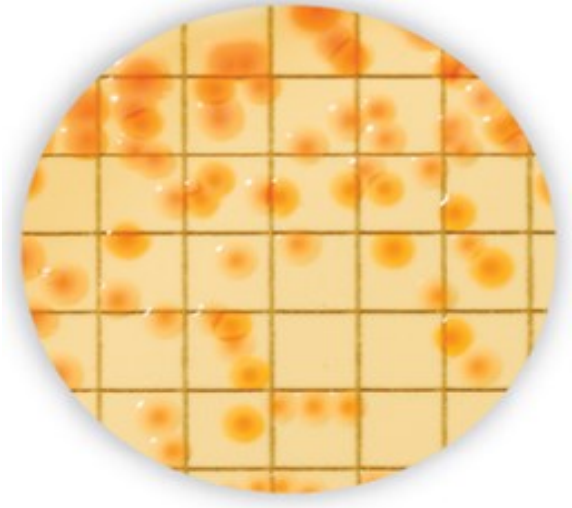


Figure 2 – E. coli on TTC Tergitol 7

Research and enumeration of intestinal Enterococci

The technique used in this analysis is membrane filtration.

Technique:

- Sterilize the graduated stainless steel funnel and the porous membrane with a bunsen burner.
- Cool them immediately afterwards with the water to be analyzed or with sterile distilled water.
- Aseptically install a 0.45 μm nominal porosity membrane between the porous membrane and the funnel using a sterile forceps.
- Then aseptically deposit 100 to 250 ml of water to be analyzed in front of a bunsen burner.
- Then activate the vacuum pump to let the water through the membrane.
- Remove the funnel and immediately and aseptically transfer the membrane using sterile forceps to the surface of previously prepared SLANETZ and BARTLEY agar.
- The latter will be incubated at 36 ± 2 °C for 44 ± 4 hours.

After incubation, faecal Streptococci colonies appear on the medium giving red or brown color.

Reading and interpretation

After the specified incubation period, intestinal Enterococci or "D" group Streptococci appear as small pigmented colonies in red, brown.

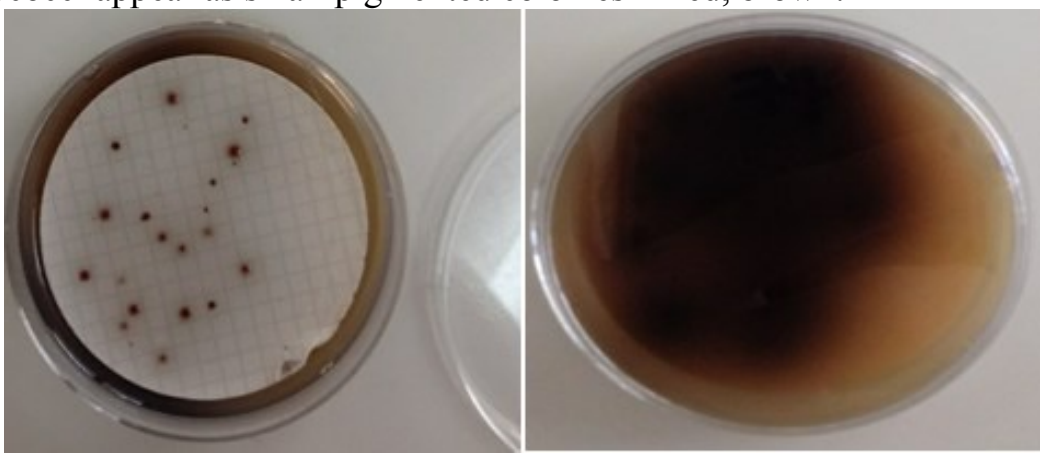


Figure 3 – Appearance of intestinal Enterococci on BEA

Confirmation test

Aseptically transfer the membrane from Slanetz and Bartley medium to Bile esculin azide (BEA) agar. The latter will be incubated at 44 ± 0.5°C for 2 hours.

The characteristic colonies then take on a black color, thus reflecting the hydrolysis of the esculin present in the medium.

Research and Counting of Spores of Anaerobic Sulfite-Reducing Bacteria

The method used is agar incorporation in deep tubes; it is a method that allows the research and enumeration of spores of sulfite-reducing anaerobic bacteria in water by incorporation into agar deep tubes.

Technique

From the water to analyze:

- Transfer about 20 ml into a sterile tube.
- Heat the tubes in a water bath of about 80 °C for 10 minutes, in order to destroy all the vegetative forms of the anaerobic sulphite-reducing bacteria possibly present.
- Cool immediately under running water.
- Then divide the contents of this tube into 4 different, sterile tubes at a rate of 5 ml per tube.
- Add about 18 to 20 ml of Liver Meal Agar.
- Mix the medium and the inoculum gently avoiding the introduction of air bubbles and oxygen.
- Allow to solidify on bench for approximately 30 minutes then incubate at 36 ± 2 °C for 44 ±4 hours.

Reading and interpretation:

- Playback will be at 44 ± 4 hours.
- Count any black colony 0.5 mm in diameter having grown in bulk and report the total number of colonies in the four tubes to 20 ml of water to be analyzed.

Figure 4 – Appearance of Spores of Anaerobic Sulfite-Reducing Bacteria on Liver Meal Agar

Research of Salmonella

In this analysis, the filtration method consists of the search and identification of Salmonella present in water intended for human consumption by filtration.

Technique

The search for Salmonella by membrane filtration is carried out according to the following steps:

- Sterilize the graduated stainless steel funnel and the porous membrane with a bunsen burner.
- Cool the graduated funnel immediately afterwards with the water to be analyzed if it is available in sufficient quantity or with sterile distilled water.
- Aseptically place a 0.45 μm membrane between the porous membrane and the funnel using a sterile forceps.
- Then deposit 500 ml or more according to the availability of water to be analyzed in front of a bunsen burner.
- Then activate the vacuum pump to absorb water through the membrane.
- Remove the membrane with a sterile forceps and place in a vial containing the SFB double concentration medium and add 20 SFB additive discs.
- Mix the filter well in the medium and incubate it at 37 °C for 24 to 48 hours. This step is the primary enrichment.
- After incubation, carry out a secondary enrichment by transferring 1 ml of the primary enrichment to the SFB medium in tube (double concentration = D / C).
- Mix the medium well with the inoculum and incubate at 37 °C for 20 ± 4 hours.
- After incubation, isolate on Hektoen medium and incubate at 36 ± 2 °C for 20 ± 4 hours.

Reading and interpretation:

- Identify characteristic colonies.
- Make a biochemical identification mainly based on ONPG, TSI, Urea-Indole, LDC...
- If necessary, make an antigenic identification based essentially on agglutination.

Figure 5 – Appearance of Salmonella on Hektoen

Research of *Staphylococcus aureus*

In this analysis, the membrane filtration method consists of the research and enumeration of coagulase positive staphylococci.

Technique

The research of coagulase-positive Staphylococci or more particularly *Staphylococcus aureus* by membrane filtration which proceeds according to the following steps:

- First, the graduated stainless steel funnel and the porous membrane should be sterilized with a bunsen burner.
- Cool them immediately afterwards with the water to be analyzed if it is available in sufficient quantity or with sterile distilled water.
- Aseptically install a 0.45 μm porosity membrane between the porous membrane and the funnel using a sterile forceps.
- Fix this device with the corresponding clamp.
- Then aseptically deposit 100 or 250 ml of water to be analyzed, depending on the type of water to be analyzed in front of a bunsen burner.
- Then activate the vacuum pump to absorb water through the membrane.
- Remove the funnel and immediately and aseptically transfer the membrane using a sterile round-nosed forceps to the prepared Chapman mannitol agar surface.
- The latter will be incubated at 36 ± 2 °C for 44 ± 4 hours.
- Reading and interpretation
- After the incubation period, coagulase-positive Staphylococci, or more particularly Staphylococcus aureus, appear as small colonies pigmented in yellow (fermentation of mannitol).
- Take 3 to 5 colonies, one half colony will be used for the catalase test, the other half will be triturated in a tube containing BHIB broth, incubate at 36 ± 2 °C for 20 ± 4 hours.

Catalase test

- Separately place two drops of a 20 volume hydrogen peroxide solution on a microscope slide. Take a half colony with a glass rod (Pasteur pipette) and gently emulsify it in one of the two drops.
- Observe immediately and after 5 minutes whether there is appearance (catalase positive) or absence (catalase negative) of oxygen bubbles.
- The observations can be made macroscopically or using a microscope at low magnification.

Coagulase test:

- After incubation of the BHIB broth, add 0.1 ml of this culture sterile to 0.3 ml of rabbit plasma contained in a sterile haemolysis tube, and incubate again at 36 ± 2 °C for 2–6 hours.
- Examine coagulation of rabbit plasma otherwise re-incubate and examine again at 20 ± 4 hours.
- Consider that the reaction to coagulase is positive when the coagulum occupies more than three quarters of the volume initially occupied by the liquid.

Figure 6 – Coagulase positive (*S. aureus*)

Figure 7 – Appearance of *S. aureus* on Chapman

Research of *Pseudomonas aeruginosa*

The method used in this analysis is the membrane filtration method.

Technique

The search for *Pseudomonas aeruginosa* by membrane filtration requires prior preparation which takes place according to the following steps:

- Sterilize the graduated stainless steel funnel and the porous membrane with a bunsen burner.
- Cool them immediately afterwards with the water to be analyzed if it is available in sufficient quantity or with sterile distilled water.
- Aseptically install a 0.45 µm nominal porosity membrane between the porous membrane and the funnel using a sterile forceps.
- Then aseptically deposit 100 or 250 ml of water to be analyzed, depending on the type of water to be analyzed, in front of a bunsen burner.
- Then activate the vacuum pump to absorb water through the membrane.
- Remove the funnel and immediately and aseptically transfer the membrane using a sterile forceps to the surface of a previously prepared cetrimide agar plate.
- The latter will be incubated at 36 ± 2 °C for 44 ± 4 hours.

Reading and interpretation

After the specified incubation period, Green blue pigmented colonies thus producing pyocyanin are considered colonies of *Pseudomonas aeruginosa.*

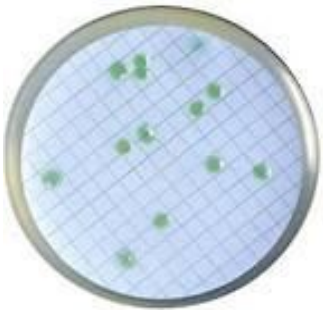


Figure 8 – *P. aeruginosa* on Cetrimide

Figure 9 – Identification of *P. aeruginosa* -API 20NE-

Research of *Vibrio cholerae*

The method used to search for this germ is as follows:

- In a bottle containing 50 ml of EPA (Alkaline Peptone Water), add 450 ml of the water sample to be analyzed (seawater case) under sterile conditions.

- Incubation 8 hours at 37 °C.

The first isolation: without shaking, using a graduated pipette and a pro pipette flambéed from the surface, a drop is collected and seeded on a petri dish (GNAB agar) and then incubated at 37 °C / 24 hours, and 1 ml of the stock solution is also deposited in a tube containing EPA, incubated at 37 °C / 24 hours.

Second isolation: from one drop from the tube onto another petri dish (GNAB agar),incubate at 37 °C / 24 hours.

Reading and interpretation:

- Aspect of the tube: homogeneous disorder.
- On GNAB: characteristic colonies: round, medium size, about 2 mm in diameter after 18 hours of culture, bluish translucent with regular edges. But the appearance of colonies varies greatly depending on the seeded peptone: presence of *Vibrio cholera*.
- Not agglutinating or clumping.

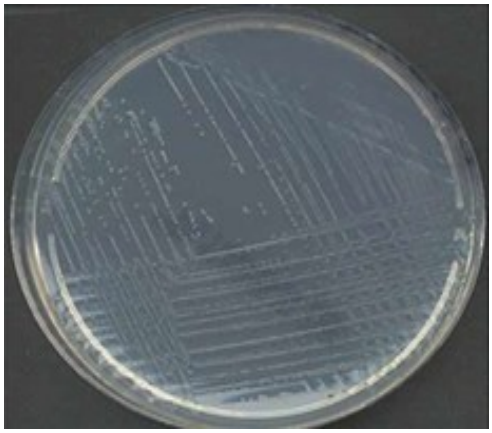


Figure 10 – *V. cholerae* on GNAB

Conclusion

Although it is now possible to detect the presence of many pathogens in water, the methods of isolation and enumeration are often complex and time-consuming. A more logical approach is the detection of organisms normally present in the faeces of man and other warm-blooded animals as indicators of excremental pollution, as well as of the efficacy of water treatment and disinfection. The presence of such organisms indicates the presence of faecal material and thus of intestinal pathogens. (The intestinal tract of man contains countless rod-shaped bacteria known as coliform organisms and each person discharges from 100 to 400 billion coliform organisms per day in addition to other kinds of bacteria). Conversely, the absence of faecal commensal organisms indicates that pathogens are probably also absent. Search for such indicators of faecal pollution thus provides a means of quality control. The use of normal intestinal organisms as indicators of faecal pollution rather than the pathogens themselves is a universally accepted principle for monitoring and assessing the microbial safety of water supplies. Ideally, the finding of such indicator bacteria should denote the possible presence of all relevant pathogens.

References

1. Chauret C. [et al.]. Detection of *Aeromonas hydrophila* in a drinking-water distribution system: a field and pilot study. *Can. J. Microbiol*. 2001; vol. 47:782–786.
2. Clausen E. M. [et al.]. Fecal streptococci: indicators of pollution. *American Society for Testing and Materials, ASTM STP*. 1977; no. 635:247–264.
3. Delarras C. [et al.]. *Surveillance sanitaire et microbiologique des eaux : réglementation, prélèvements, analyses.* Editions Tec et Doc-EM Inter., 2003.
4. Delarras C. [et al.]. *Surveillance sanitaire et microbiologique des eaux*. Lavoisier TEC & Doc., 2003.
5. Delarras C. [et al.]. *Microbiologie pratique pour le laboratoire d'analyses ou de contrôle sanitaire*. Editions Tec et Doc-EM Inter-Lavoisier, 2007.
6. Delay M. [et al.]. *La gestion des aquifers*. MASSON, 1997.
7. Delcourt M. O. [et al.]. *Equilibres chimiques en solution*. De Boeck Université, 2000.
8. Desjardins C. [et al.]. *Le traitement des eaux*. Lavoisier TEC & Doc., 1997.
9. Desjardins R. [et al.]. *Le traitement des eaux*. Presses Internationales Polytechniques, 2e éd., 1997.
10. Edberg S. C. [et al.]. *Escherichia coli*: the best biological drinking water indicator for public health protection. *Journal of Applied Microbiology*. 2000; vol. 88:106–116.
11. Elmund G. K. [et al.]. Comparison of Escherichia coli, total coliform and fecal coliform populations as indicators of wastewater treatment efficiency. *Water Environment Research*. 1999; vol. 71:332–339.
12. El-Taweel G. E. [et al.]. Microbiological quality of drinking water at eight water treatment plants. *Int. J. Environ. Health Res*. 2001; vol. 11:285–290.
13. Gleeson C. [et al.]. The coliform index and waterborne disease. E & FN Spoon, 1997:194.
14. Haslay C. [et al.]. *Microbiologie des eaux d'alimentation*. Technique et Documentation, 1993.
15. Larpent J. P. [et al.]. *Mémento technique de microbiologie*. Technique et Documentation, 1990.
16. Leclerc H. [et al.]. Advances in the bacteriology of the coliform group: their suitability as markers of microbial water safety. *Annu. Rev. Microbiol*. 2001; vol. 55:201–234.
17. Martin G. [et al.]. Point sur l'épuration et le traitement des effluents (Eau/Air). *Tec et Doc. Bactériologie des milieux aquatiques*. 1985; vol. 2.
18. Olivieri V. P. [et al.]. Bacterial indicators of pollution. *Bacterial indicators of pollution*. CRC Press, 1982:21–41.
19. Rodier J. [et al.]. *L'Analyse de l'eau*. Dunod 9e edition, 2009: 1579 p.

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