Microbiological parameters of drinking water

III Definition of indicator organism and drinking water quality standards

Water may be contaminated with high numbers of microorganisms and, thus, might pose a risk of diseases. To control the safety of drinking water, it is regularly tested for specific indicator organisms, the presence of which signals the potential for waterborne diseases [3].

Many criteria are described that characterize the ideal test organism, including that [1;3]:

1. The organism should be useful for all types of water.

2. The organism should be present whenever enteric pathogens are present.

3. The organism should have a reasonably longer survival time than the hardiest enteric pathogen.

4. The organism should not grow in water.

5. The testing method should be easy to perform.

6. The density of indicator organism should have some direct relationship to the degree of faecal pollution.

7. The organism should be a member of the intestinal microbiota of warm-blooded animals. A marker reflecting the general microbiological condition of food or environment is defined as an "indicator organism" in microbiological testing [6]. The coliform group, which includes *Escherichia*, *Citrobacter*, *Enterobacter* and *Klebsiella* species, is aerobic and facultatively anaerobic, Gram-negative, non-spore-forming, rod-shaped bacteria that produce gas upon lactose fermentation in prescribed culture media within 48 hours at 35° C. Coliforms have been used as indicator organisms since early 20th century. To begin with, they were used in evaluation of water for faecal contamination, thereafter, in identifying unsanitary conditions in different types of food and water samples [4].

Bacterial indicators such as coliforms have been used for the development of water quality standards. The use of microbial standards also requires the development of standard methods and quality assurance, or quality controls plans for the laboratories that conduct the monitoring. Knowledge of how to sample and how often to sample is important. All this information is usually defined in the regulations when a standard is set [2;3].

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Table 1 summarizes the data about the microbiological criteria of drinking water of the European Union. The EU Directive 80/778/EEC recommends total coliforms, faecal coliforms and faecal streptococci to be evaluated. Differently, the new CD 98/83/EC requires *E. coli* and enterococci to be determined. Indicators are among parameters found most frequently at levels of concern in the drinking water of many European countries [7].

If it is necessary, samples of water intended for human consumption could be examined also for the following pathogens as *Salmonella*, pathogenic staphylococci, faecal bacteriophages and enteroviruses. Moreover, such water should not contain parasites, algae and other organisms [7].

Table 1. Drinking Water Microbiological Criteria of the European Union

Indicator	**Parametric value**
Tap water	
Escherichia coli	0/100 mL
Faecal streptococci	0/100 mL
Sulfite-reducing clostridia	0/20 mL
Bottled Water	
Escherichia coli	0/250 mL
Faecal streptococci	0/250 mL
Sulfite-reducing clostridia	0/50 mL
Pseudomonas aeruginosa	0/250 mL
Total count 22°C	100/mL
Total count 37°C	20/mL

Escherichia coli can be easily distinguished from other members of the faecal coliform group by absence of urease and presence of β-glucuronidase, and is more likely to indicate faecal pollution.

The faecal streptococci belong to the genera *Enterococcus* and *Streptococcus* [8]. The enterococci can be found in soil, water, dairy products, food and plants. They are differentiated from other streptococci by their ability to grow in 6.5 % sodium chloride, pH 9.6. and 45° C. Of the genus *Streptococcus*, only *S.bovis* and *S.equinus* are considered to be true faecal streptococci. Enterococci are considered to have certain advantages over the coliform and faecal coliform bacteria as indicators: the rarely grow in water; they are more resistant to environmental stress and chlorination than are coliforms; they generally persist longer in the environment [3;5].

Clostridium perfringens is a sulfite-reducing anaerobic spore former; it is Gram positive, rod shaped and exclusively of faecal origin. The spores are very heat resistant, persist for long periods in the envirnoment and are very resistant to disinfectants [3].

Drinking water microbiological criteria are very similar in all European Union countries, including Latvia. Table 2 summarizes the data for Regulations of the Cabinet of Ministers No. 671 "Minimum safety and quality requirements for drinking water, monitoring and control procedures".

Table 2. Drinking Water Microbiological Criteria of Latvia.

Indicator	**Parametric value**
Tap water:	
Escherichia coli	0/100 ml
enterococci	0/100 ml
Bottled Water	
Escherichia coli	0/250 ml
enterococci	0/250 ml
Pseudomonas aeruginosa	0/250 ml
Colony forming units (CFU) 22°C	100/ml
Colony forming units (CFU) 37°C	20/ml

The Regulations include legal norms arising from Council Directive 98/83 / EC of 3 November 1998 on the quality of water intended for human consumption and Commission Directive (EU) 2015/1787 of 6 October 2015 amending Annexes II and III to Council Directive 98/83 / EC on the quality of water intended for human consumption.

Microbiological analyses are conducted according to scientific methods laid down in the Annex III of the directive (table 3). However, other methods can be used providing that results are equivalent to or comparable with those obtained by the methods above mentioned.

Table 3. Methods of analysis for microbiological parameters (CD 80/778/EEC, Annex III)

Parameter	**Method**
Total coliforms (*) Faecal coliforms (*)	Fermentation in multiple tubes. Subculturing of the positive tubes on a confirmation medium. Count according to Most Probable Number or Membrane filtration and culture on an appropriate medium such as Tergitol lactose agar, endo agar, 0.4 % Teepol broth, sub-culturing and identification of the suspect colonies Incubation temperature for total coliforms: 37 °C Incubation temperature for faecal coliforms: 44 °C
Faecal streptococci (*)	Sodium azide (Litsky). Count according to MPN Membrane filtration and culture on an appropriate medium
Sulphite-reducing *Clostridia* (*)	A spore count, after heating the sample to 80 °C by: • Seeding in a medium with glucose, sulphite and iron, counting the black-halo colonies • Membrane filtration, deposition of the inverted filter on a medium with glucose, sulphite and iron covered with agar, count of black colonies • Distribution in tubes of differential reinforced clostridial medium (DRCM), sub-culturing of the black tubes in a medium of litmus-treated milk, count according to MPN
Total counts (*)	Inoculation by placing in nutritive agar

(*) incubation period is generally 24 or 48 hours except for total counts, when it is of 48 or 72 hours.

Self-control questions.

1. What is a test organism? What qualities should it possess?
2. What are the common features of coliform group bacteria?
3. Using additional sources of information, find out which documents regulate drinking water safety rules in your country/region.
4. Why it is necessary to count total number of bacteria in a sample at 37°C temperature?

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