

Report of the Scientific Committee
of the Food Safety Authority of Ireland

2009

The consumption of bottled water containing certain bacteria or groups of bacteria and the implications for public health



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Published by:
Food Safety Authority of Ireland
Abbey Court, Lower Abbey St
Dublin 1

Advice Line: 1890 336677
Tel: +353 1 8171300
Fax: +353 1 8171301
info@fsai.ie
www.fsai.ie

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ISBN 1-904465-63-3

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Background

A 2007 study of the microbiological safety and quality of bottled water on sale in Ireland found that 7.2% of bottled water tested failed to meet European microbiological standards¹ or guidelines² (FSAI, 2008). This study raised a question as to the food safety risks posed by such water so in January 2009, the FSAI requested its Scientific Committee to provide an opinion on the risks posed by bottled waters contaminated with one or more of the following bacteria or groups of bacteria:

- *Escherichia coli*
- enterococci
- coliforms
- *Pseudomonas aeruginosa*.

In addition, the Committee was requested to consider, in each case, if there was a correlation between the numbers of these bacteria detected in bottled water and any risks to public health.

Introduction

Bottled water is any potable water offered for sale in a sealed container. Three types of bottled water are defined in Irish legislation (S.I. No. 225 of 2007 as amended by S.I. No. 686 of 2007). They are:

1. natural mineral water (NMW)
2. spring water (SW) and
3. other waters (OW).

Bottled waters do not include waters which are medicinal products.

The National Standards Authority of Ireland (NSAI) define NMW and SW as microbiologically wholesome waters, originating in an underground water table or deposit and emerging from a spring, tapped at one or more natural or bore exits and packaged at source (NSAI, 2005). Furthermore, NMW is clearly distinguishable from other drinking water by its nature (i.e. its content of minerals and trace elements) and by its original state. It is bottled at source and is certified as a NMW by the responsible authority (NSAI, 2005). In Ireland, the responsibility to certify NMW lies with the NSAI. Water, other than medicinal water, which is intended for human consumption and is placed on the market in either bottles or containers but is neither a NMW nor a SW, falls into the category of OW.

There are currently 24 plants engaging in bottling water that are registered in Ireland.

¹ Microbiological standards are limits for bacteria in foods that are established by food legislation.

² Microbiological guidelines are limits for bacteria in foods that are advisory but are not established by food legislation. They are limits that are achievable by adherence to good hygienic practice and good manufacturing practice.

Dynamics of Microbial Populations in Bottled Water

The microbiological characteristics of groundwater and surface water are somewhat different. The filtration of water as it seeps through the sub-soil and underlying rock tends to exclude organisms larger than microorganisms and influences the microbial content of the water. Also, the subterranean environment is devoid of light and ground water has a different gaseous and mineral composition to surface water. Consequently, these factors affect the nature and composition of microbiological communities contained in water.

Natural mineral waters and spring waters derive from underground sources. The metabolism of bacteria in underground waters is primarily heterotrophic and depends on dissolved minerals. The major group of bacteria is the aerobic Gram-negative rods characterised by the presence of cytochrome oxidase. Eukaryotic organisms may be absent or only present in small numbers, while cyanobacteria and algae are not found (Leclerc and Moreau, 2002). Subsurface water contains little organic matter and is an oligotrophic environment for bacteria. Hence, bacteria from the genus *Pseudomonas* are common in groundwater as are bacteria of the genus *Acinetobacter* and the genus *Alcaligenes*. Other groups like *Caulobacter*, *Cytophaga*, *Flexibacter* and *Flavobacteria* are also widely distributed but may predominate in water from shallow aquifers (Leclerc and Moreau, 2002).

European legislation does not allow the treatment of natural mineral waters and spring waters in order to remove microorganisms. Other bottled waters may receive treatment and when this is practiced it often consists of ozonation, ultraviolet treatment or filtration rather than chlorination. Additionally, there is no legislative restriction on manufacturers bottling tap water that has been previously chlorinated by the local authority. However, it is generally the case that most bottled water is untreated and will contain a natural microflora (autochthonous bacteria).

“ European legislation does not allow the treatment of natural mineral waters and spring waters in order to remove microorganisms ”

Because of the nutrient-depleted environment of water, these microorganisms are adapted to starvation conditions allowing them to survive for long periods of time (Leclerc and Moreau, 2002). However, when water is bottled by compressed air it alters the environment that is encountered by these microorganisms, because oxygenation of the water increases and nutrients can deposit on bottle surfaces, effectively concentrating them and making them more available for microorganisms to use. This is one possible explanation for the observed phenomenon, whereby, the number of microorganisms in bottled water increases after bottling (ZoBell and Anderson, 1936). However, it is also possible that there is an increase in culturable bacteria in bottled water due to the resuscitation of a large number of viable but non-culturable cells (VBNC) rather than being due to growth or multiplication of a few cells already present at the point of bottling (Leclerc and Moreau, 2002). Whatever the explanation, the general increase in numbers of microorganisms after bottling is not necessarily due to contamination of the water during bottling.

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In summary, the natural bacteriological community of natural mineral water after bottling has been shown to be diverse and complex (Loy *et al*, 2005). Clearly therefore, a variety of microorganisms are likely to be present in natural mineral waters, spring waters and untreated other waters. Accordingly, it is important to communicate to the public that “the detection of bacteria” in bottled water is not in itself a cause for concern in the absence of evidence of the presence of certain bacteria used as an “index” of faecal contamination (see ‘Microbiological Standards for Bottled Water’ on page 5 for an explanation of the term “index”).

Outbreaks of Human Disease Attributable to Bottled Water

From time to time, bacteria may be present in bottled waters at low concentrations. Usually, these bacteria are harmless and reports of outbreaks of human illness linked to bottled water are infrequent in the international literature compared to reports of outbreaks of human illness linked to tap water.

In 1974, there was an outbreak of cholera associated with bottled mineral waters in Portugal. There were 2,467 bacteriologically confirmed cases, of which, 48 died. Bottled water was not the only cause of the outbreak; however, 82 patients had a history of drinking bottled mineral water from one particular source. A further 36 cases had visited the spa served by the same water source as the bottled water. *Vibrio cholerae* was found in the water source (Blake *et al*, 1977a,b: cited in Hunter, 1993).

In 2006, there was a reported outbreak of *Salmonella enterica* serovar Kottbus in infants in Gran Canaria. Forty six cases were identified and 41 of which were studied. The average age of cases was five months. Nineteen cases had underlying disease or were immunocompromised. The case control analysis identified a statistically significant association with the consumption of bottled water and natural fruits. *Salmonella* Kottbus was isolated from bottled water randomly selected from the markets and in the local factory where the water was bottled (Palmera-Suarez *et al*, 2007).

Eckmanns *et al*. (2008) described an outbreak of hospital-acquired *P. aeruginosa* infection caused by contaminated bottled water in intensive care units in a hospital in Germany. Contaminated bottled water was used for the preparation of orally administered medications and oral fluid replacement. Unopened bottles of water were found to contain the outbreak strain of *P. aeruginosa* although not all bottles were contaminated. The authors concluded that bottled water should not be used in intensive care units unless sterilised (Eckmanns *et al*, 2008)

In Saipan, Northern Mariana Islands, an outbreak caused by a non-O1 *Vibrio cholerae* was associated with contaminated bottled water and caused illness in 11 persons, four of whom were hospitalised. Because public tap water in most locales in Saipan was too salty to be drinkable, two commercial bottled-water plants treated municipal water with reverse osmosis to supply drinking water to the public. Although the bottles were to have been cleaned by machine or manually with hot water and a chlorine solution, the bottling plants had occasionally been cited for the cursory handling of returned bottles, e.g. for only rinsing them with treated water. During the outbreak period, bottled water tested positive for faecal coliforms. The source of the contamination was not determined (CDC, 1996).

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In 1997, an outbreak of *Campylobacter jejuni* was reported among 106 guardsmen of the Minnesota Army National Guard following a training exercise in Greece. The source of the outbreak was bottled water consumed during international field exercises. The water was bottled in Greece (CDC, 2000).

A multistate outbreak of *S. Bareilly* was detected through CDC's *Salmonella* Outbreak Detection Algorithm (SODA). Epidemiology implicated the consumption of bottled water as a risk factor for illness (CDC, 2002).

Microbiological Standards for Bottled Water

The microbiological safety and quality of bottled water is of paramount importance considering it is consumed by all sectors of the population without further treatment. Its safety and quality is influenced by a number of factors including the microbiological status of the source water and the level of hygiene control in the extraction and bottling process. The legal microbiological standards that apply to bottled waters are provided in Annex 1. However, sampling plans associated with these standards are not mandated in the legislation. Bacteria tend to be log-normally distributed in food and water and therefore the information provided by testing a single sample is minimal and provides little assurance that all bottles in the batch are compliant with the standards (Jarvis, 2000). The use of statistical sampling plans is common throughout the wider food industry to address this issue.

Certain microorganisms can be used to signal the potential presence of pathogenic microorganisms. An 'index' is a microorganism or group of microorganisms which, if detected, points to the presence of pathogenic organisms. An 'indicator' is a microorganism or group of microorganisms which, if detected, is a measure of the effectiveness of a process (World Health Organization (WHO), 2006). It is considered that monitoring water for indicator and index microorganisms protects public health more effectively than monitoring for specific pathogens (Edberg *et al*, 2000). There are many recognised waterborne pathogens; when they occur they are difficult to detect as in many such cases they are present in low numbers. Bacteria used either as an index or an indicator of faecal pollution should, ideally, universally be present in faeces of humans and other mammals, be present in large numbers, be readily detected by simple inexpensive methods and should not multiply once they have left the body and entered water (Edberg *et al*, 2000).

“ It is considered that monitoring water for indicator and index microorganisms protects public health more effectively than monitoring for specific pathogens ”

Risks Associated with the Detection of Index and Indicator Organisms in Bottled Water

1. *E. coli* in Bottled Water

Brief description of the organisms

E. coli are oxidase-negative Gram-negative rods that are usually motile. They have an optimum growth temperature of 37°C and a growth temperature range of 15°C to 45°C. *E. coli* are facultative anaerobes that ferment glucose with the production of acid and gas, produce indole from tryptophan and produce beta-glucuronidase (although up to 10% may not) (Ashbolt *et al*, 2001). *E. coli* are readily isolated and measured using standard microbiological methods (ISO 9308-1). Most *E. coli* are commensal bacteria; that is to say they are unlikely to be associated with disease when ingested by healthy people. However, some strains of *E. coli* may be associated with disease and these strains can be distinguished from commensal, i.e. non-pathogenic, strains by using a variety of phenotypic and molecular methods.

There are four main categories of *E. coli* that are associated with foodborne disease. These are the:

- verocytotoxigenic strains (VTEC)
- enterotoxigenic strains (ETEC)
- enteroinvasive strains (EIEC)
- enteropathogenic strains (EPEC).

The most important sub-group of VTEC are the enterohaemorrhagic strains (EHEC) as these may be associated with bloody diarrhoea and haemolytic uraemic syndrome (HUS).

Other categories of *E. coli* such as enteroaggregative strains (EA_gEC) and diffusely adherent strains (DAEC) are also recognised, but their virulence mechanisms are less well understood. Interestingly, EA_gEC, were found to survive in bottled and spring water for 60 days at temperatures of 4°C, 10°C and 23°C (Vasudevan *et al*, 2003).

The health significance of the detection of *E. coli* in bottled water

E. coli is a bacterium which occurs in the faeces of all mammals. *E. coli* does not multiply appreciably in the environment and can survive in drinking water at 15°C to 18°C for between four and 12 weeks (Edberg *et al*, 2000). Consequently, *E. coli* is regarded as the primary index of faecal contamination of water (WHO, 2006; EAUK, 2002; Schindler *et al*, 1995). When *E. coli* are isolated from water using standard methods, no differentiation is made between pathogenic and non-pathogenic *E. coli*. *E. coli* are rarely found in water in the absence of faecal pollution (WHO, 2006). Therefore, the presence of *E. coli* in bottled water is indicative of recent faecal contamination of the water.

Generally, there is no direct correlation between the number of *E. coli* present in contaminated water and the number of enteric pathogens (Granbow, 1996). Rather, the detection of *E. coli* in bottled water indicates that there is the likelihood that bacterial pathogens derived from faeces are present also. It

does not confirm that pathogens are actually present (Ashbolt, 2001). In addition, failure to detect *E. coli* does not mean that the water is free from viruses or protozoa like *Cryptosporidium* spp. (Kramer *et al*, 1996; Edberg *et al*, 2000).

Water that is found to contain *E. coli* must be considered unsafe for consumption due to the strong association between *E. coli* and faecal contamination.

2. Enterococci in Bottled Water

Brief description of the organism group

Bacteria of the genus *Enterococcus* were formerly referred to as the faecal streptococci or Lancefield group D streptococci. They are Gram-positive cocci that are facultative anaerobes displaying alkaline and salt tolerance (WHO, 2006). The *Enterococcus* genus consists of a number of species of which *E. faecalis* and *E. faecium* are predominant (Edberg *et al*, 2000). Nearly all animals carry enterococci and shed them in their faeces, usually at lower concentrations than *E. coli*.

The health significance of the detection of enterococci in bottled water

Most enterococci do not multiply in water but tend to survive in water longer than coliforms and most enteric pathogens (Warburton, 1993). Their utility as an index of faecal pollution of water is subject to differing opinion. In its 2002 report, the Environment Agency, UK (EAUK) suggested that they are only secondary indicators of faecal pollution to be used to assess the significance of the presence of coliform bacteria in the absence of *E. coli* (EAUK, 2002). However, the more recent WHO drinking water report in 2006 considered that enterococci are an index of faecal pollution serving as a marker for faecal pathogens that survive longer in water than *E. coli* (WHO, 2006). Therefore, the detection of enterococci in water is an index of faecal pollution even when *E. coli* and/or coliforms are not detected, because of the better survival of enterococci in water compared to these other bacteria.

Ingestion of *Enterococcus* spp. is not associated with gastrointestinal disease although enterococci can be significant pathogens in the urinary tract and at other sites, particularly in patients with predisposing conditions.

There are no reports of a correlation between the numbers of enterococci present in contaminated water and the numbers of enteric pathogens. Therefore, like *E. coli*, detection of enterococci in bottled water indicates that there is the likelihood that bacterial pathogens derived from faeces are present also. However, it does not confirm that pathogens are actually present.

Water containing enterococci should be considered unsafe due to the strong association between enterococci and faecal contamination.

“Water that is found to contain *E. coli* must be considered unsafe for consumption due to the strong association between *E. coli* and faecal contamination.”

3. Coliforms in Bottled Water

Brief description of the organism group

Coliform bacteria (or total coliforms) belong to the family Enterobacteriaceae. Typical genera belonging to the coliform group found in water include *Citrobacter*, *Enterobacter*, *Escherichia*, *Hafnia*, *Klebsiella*, *Serratia* and *Yersinia* (EAUK, 2002). These bacteria utilise lactose at 37°C to produce acid and gas, possess the enzyme beta-D-galactosidase and are oxidase negative (EAUK, 2002). Coliforms include bacteria that are aerobic or facultatively anaerobic and also include species of bacteria that are both of faecal or environmental origin. A sub-set of the coliforms are the thermotolerant coliforms that are able to ferment lactose at 44-45°C. These include *E. coli* as well as some species of *Citrobacter*, *Klebsiella* and *Enterobacter* (WHO, 2006).

The health significance of the detection of coliforms in bottled water

Coliform bacteria are not exclusively of faecal origin. Although they were used traditionally as indicators of water quality because testing methodology was simple and inexpensive, recent improvements in methods for detection of *E. coli* and enterococci have overcome this traditional advantage (Edberg *et al*, 2000). As coliform bacteria are susceptible to effective chlorination of water, the total coliform test can be used in the case of drinking water, to indicate the effectiveness of the chlorination process. However, this is generally not relevant in the case of bottled water.

The presence of coliforms in bottled water is not necessarily indicative of faecal contamination of the water (WHO, 2006). Some species can also grow in water and therefore they do not mimic enteric pathogens in this respect. Coliform bacteria also originate in the environment and can form biofilms on bottling equipment. Therefore, coliforms may indicate a problem with the quality of the water source or indicate possible contamination during the bottling process.

Although many species of coliform have been associated with hospital-acquired infections in vulnerable patients, they do not, in general, represent a direct risk to public health. Therefore, whilst their presence in bottled water samples is significant from a hygiene perspective, from a public health perspective, their presence needs to be treated with caution in the absence of bacteria like *E. coli* and enterococci that are considered the index of faecal pollution.

The presence of coliform bacteria in bottled water in isolation is not, of itself, a strong indication of potential presence of enteric pathogens.

“ The presence of coliform bacteria in bottled water in isolation is not, of itself, a strong indication of potential presence of enteric pathogens. ”

4. *P. aeruginosa* in Bottled Water

Brief description of the organism

Pseudomonads are part of the normal microflora of bottled water. They inhabit the environment in soils, ground water, marine environments and plants. Bacteria of this genus are straight or slightly curved rod-shaped Gram-negative cells (Leclerc and Costa, 2005). Pseudomonads are usually strict aerobes and cannot grow when oxygen levels are low. Members of the genus can grow in low nutrient environments. The most important species in mineral waters are the fluorescent and non-fluorescent pseudomonad species (Leclerc and Moreau, 2002).

In contrast to other pseudomonads, *Pseudomonas aeruginosa* is not a normal component of the microbiological flora of natural mineral waters (Leclerc and Moreau, 2002). When it is detected it is usually in low numbers but *P. aeruginosa* can grow and survive in natural mineral water. Legnani *et al.* (1999) studied two strains of *P. aeruginosa* and found that counts increased from an inoculated density of 10^2 cfu/ml by 3 log units after four to five days. This increased level was maintained between 70 and 100 days after inoculation and decreased slowly, culminating in the death of one strain after five years. Pseudomonads including presumptive *P. aeruginosa* have been shown to colonise bottled water plants on a persistent basis (Morais *et al.*, 1997).

The health significance of the detection of *Pseudomonas aeruginosa* in bottled water

Contact with *P. aeruginosa* in contaminated bathing water may be associated with superficial or local infections, e.g. external ear infections. However, the vast majority of people exposed to *P. aeruginosa* suffer no adverse health effects (EAUK, 2002). Buck and Cooke (1969) found that colonisation of healthy human volunteers by *P. aeruginosa* required an oral dose in excess of 1.5 million bacteria. *P. aeruginosa* generally does not infect normal tissue and it appears that only certain hosts are at risk; these include patients with profound neutropaenia, cystic fibrosis and severe burns (Allen *et al.*, 2004). *P. aeruginosa* is therefore considered an opportunistic pathogen and is associated with hospital-acquired infections in individuals that are profoundly immunocompromised (EAUK, 2002).

P. aeruginosa in bottled water can only be considered a risk to profoundly immunocompromised patients in hospitals and does not appear to be a risk for the general population.

Discussion

“Bottled water should be of better microbiological quality than most other foods especially if intended to be used by vulnerable populations” (Warburton, 1993). This statement by the author was supported by two facts. Firstly, up to 50ml water taken between meals moves immediately to the intestine via the stomach and therefore, any pathogenic bacteria, if present, rapidly escape the acidic stomach conditions that may kill them (Mossel and Oei, 1975: cited in Warburton, 1993). Secondly, the infectious dose could be low in such circumstances. However, it is also important to note that bottled water is consumed without further treatment whereas a certain quantity of drinking water and many foods are often subjected to treatments like cooking that kill bacteria.

Microbiological standards exist in law for all types of bottled water and these should not be exceeded. Microbiological methods as specified in legislation should always be used and testing should be carried out by a laboratory that has these methods within the scope of its accreditation to ISO17025. Additionally, manufacturers should monitor their products using sampling and testing protocols that provide a good level of assurance that they are compliant with legal requirements. Single samples provide only minimum information about the contamination of water or foods particularly when the concentration of microorganisms is low. In contrast, the adoption of suitable 2 or 3-class attribute plans provides a quantifiable probability of acceptance of a batch of water given a certain level of contamination (ICMSF, 1986). Manufacturers will always be required to operate under more stringent microbiological limits than those prescribed in legislation to provide themselves with a reasonable level of assurance that their products comply with applicable food law.

In Ireland, bottled water sales have risen steadily since 2001 from 113 million litres to 193 million litres in 2007. The 2007 figure means that on average, 44.9 litres of bottled water are consumed annually per person (Beverage Council of Ireland, 2008). As a consequence of this increased consumption, there is potentially an increased likelihood of exposure to harmful bacteria if standards are not maintained during bottled water production. Additionally, as previously mentioned, the passage of any waterborne pathogenic bacteria directly to the intestine via the stomach will avoid a known barrier to human infection and therefore, this must also be considered when assessing risk. Hence, the presence of *E. coli* and/or enterococci in water poses a higher risk to public health than the presence of the same organisms in other foods.

“Manufacturers will always be required to operate under more stringent microbiological limits than those prescribed in legislation to provide themselves with a reasonable level of assurance that their products comply with applicable food law. ”

Conclusions

Bottled water that tests positive for *E. coli* or enterococci should be regarded as contaminated with faeces and therefore represents a risk to public health. Such water should be considered unsafe for consumption irrespective of the lack of direct detection of known pathogenic bacteria.

Bottled water that tests negative for *E. coli* and enterococci but positive for coliform bacteria need not be regarded as contaminated with faeces. The presence of coliform bacteria on its own is not of itself a strong indication of potential presence of enteric pathogens. Such bottled water should not be considered as unsafe for consumption but does require careful investigation of the potential causes and an enhanced monitoring of finished product and source water for the presence of *E. coli* and enterococci.

The presence of *Pseudomonas aeruginosa* in bottled water in the absence of *E. coli* and enterococci is not a significant health risk for the general population. *Pseudomonas aeruginosa* is clearly a risk for the severely immunocompromised sub-population found in certain areas of hospitals like intensive care units. However, suitable measures within hospitals can be adopted to reduce or remove this risk.

Recommendations

1. The bottled water industry and the authorities should understand that the presence of *E. coli* or enterococci in bottled water is an index of faecal contamination. The presence of these organisms in bottled water is unacceptable as it is indicative of the potential presence of enteric pathogens. Such contaminated product represents a risk to public health and appropriate action should be taken to ensure that such bottled water is not consumed.
2. The bottled water industry should ensure that the water they place on the market is free from faecal contamination. To provide this level of assurance, it is important that, as an ongoing measure, manufacturers have an effective HACCP-based system in place that includes measures necessary to protect the water source and also covers the water extraction process as well as the bottling process.
3. Manufacturers should apply statistically appropriate attribute sampling plans to the microbiological monitoring of both the water source and the bottled water in order to provide a reasonable level of assurance that their products comply with the microbiological standards set in the legislation.
4. Bottled water manufacturers should investigate all breaches of microbiological standards, categorise any breaches appropriately and take appropriate measures to ensure the protection of public health.
5. As an additional hygiene measure, manufacturers should consider applying a suitable antimicrobial treatment to bottled water if this is in accordance with legal requirements governing these products.

“Bottled water that tests positive for *E. coli* or enterococci should be regarded as contaminated with faeces and therefore represents a risk to public health.”

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Annex 1. Summary of Microbiological Criteria Pertaining to Bottled Water

Table 1: Microbiological Criteria for Bottled Natural Mineral Water and Bottled Spring Water as Specified in S.I. No. 225/2007

(European Communities (natural mineral waters, spring waters and other waters in bottles or containers) Regulations, 2007)[†]

PARAMETER	PARAMETRIC VALUE	STAGE WHEN THE CRITERION APPLIES
Parasites	Absence	At source and during marketing
Pathogenic micro-organisms	Absence	At source and during marketing
<i>Escherichia coli</i>	0/250 ml	At source and during marketing
Coliforms	0/250 ml	At source and during marketing
Faecal streptococci	0/250 ml	At source and during marketing
Sporulated sulphite-reducing anaerobes	0/50 ml	At source and during marketing
<i>Pseudomonas aeruginosa</i>	0/250 ml	At source and during marketing
Total Colony Count	Shall conform to their normal viable colony count and give satisfactory evidence of the protection of the source against all contamination	At source
	100/ml (20-22°C in 72 hours on agar-agar or an agar-gelatine mixture)	12 hour after bottling *
	20/ml (37°C in 24 hours on agar-agar)	
	Normal increase in the bacteria content which it had at source	At marketing stage ‡

* After bottling, the total colony count at source may not exceed 100/ml at 20 to 22°C in 72 hours on agar-agar or an agar-gelatine mixture and 20/ml at 37°C in 24 hours on agar-agar. The total colony count shall be measured within the 12 hours following bottling, the water being maintained at 4°C ± 1°C during this 12-hour period.

‡ At the marketing stage, the revivable total colony count may only be that resulting from the normal increase in the bacteria content which it had at source.

† Reference: Regulation 6 (Paragraphs 1 to 5) and Regulation 11 (Paragraph 2d) of S.I. No. 225 of 2007

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Table 2: Microbiological Criteria for Bottled Spring Water and Other Bottled Waters as Specified in S.I. No. 225/2007 (European Communities (natural mineral waters, spring waters and other waters in bottles or containers) Regulations, 2007) *

PARAMETER	PARAMETRIC VALUE	STAGE WHEN THE CRITERION APPLIES
<i>Escherichia coli</i>	0/250 ml	At the point at which the water is put into the bottle or container
Enterococci	0/250 ml	
<i>Pseudomonas aeruginosa</i>	0/250 ml	
Coliforms	0/250 ml	
Colony count 22°C	100/ml	
Colony count 37°C	20/ml	

In addition, bottled spring water and other bottled waters shall not be placed on the market unless they are wholesome and clean, i.e. they are free from any microorganisms and parasites and from any substances which, in numbers or concentrations, constitute a potential danger to human health.

* Reference: Regulation 12 (paragraph 1), Regulation 14 and Schedule 5 of S.I. No. 225 of 2007

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Members of the Scientific Committee

Prof. Albert Flynn, (Chair)

University College, Cork

Dr Catherine Adley

University of Limerick

Dr Paula Barry Walsh

Dept of Agriculture, Fisheries and
Food

Dr Colette Bonner

Dept of Health and Children

Prof. John Daniel Collins

University College, Dublin

Prof. Martin Cormican

University College Hospital,
Galway

Prof. Colin Hill

University College, Cork

Prof. Brian McKenna

University College, Dublin

Dr Paul McKeown

Health Protection Surveillance
Centre

Dr Terry McMahon

Marine Institute

Dr Michael O’Keeffe

formerly of Teagasc

Dr Dan O’Sullivan

Dept of Agriculture, Fisheries and
Food

Mr Ray Parle

Health Service Executive

Dr Iona Pratt

Food Safety Authority of Ireland

Prof. Michael Ryan

University College, Dublin

Members of the Microbiology Sub-committee

Prof. Martin Cormican (Chair)

University College Hospital,
Galway

Dr Catherine Adley

University of Limerick

Dr Paula Barry Walsh

Dept of Agriculture, Fisheries and
Food

Dr Tom Beresford

Teagasc

Dr Cyril Carroll

University College, Galway

Prof. Dan Collins

University College, Dublin

Ms Helen Cowman

HSE Southern Region

Dr Bill Doré

Marine Institute

Dr Geraldine Duffy

Teagasc

Dr Michael Fallon

Dept of Agriculture, Fisheries and
Food

Prof. Seamus Fanning

University College, Dublin

Dr Paul McKeown

Health Protection Surveillance
Centre

Mr David Nolan

Dept of Agriculture, Fisheries and
Food

Mr Ray Parle

HSE, Southern Region

Dr Neil Rowan

Athlone Institute of Technology

Members of the working group on bottled water

Prof. Martin Cormican

University College Hospital,
Galway

Dr Catherine Adley

University of Limerick

Ms Helen Cowman

HSE Southern Region

Dr Paul McKeown

Health Protection Surveillance
Centre

Mr Ray Parle

Health Service Executive

Acknowledgements

Secretariat to the Committee for the preparation of this report: Dr Wayne Anderson



Food Safety Authority of Ireland
Abbey Court, Lower Abbey Street,
Dublin 1

Udarás Sábháilteachta Bia na hÉireann
Cúirt na Mainistreach, Sráid na Mainistreach íocht.,
Baile Átha Cliath 1

Advice Line: 1890 336677
Telephone: +353 1 817 1300
Facsimile: +353 1 817 1301
E-mail: info@fsai.ie
www.fsai.ie