



3rd National Microbiological Survey 2007 (07NS3):

Microbiological safety and quality of bottled water

December 2008

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Executive Summary

This study was conducted in the last four months of 2007 to investigate compliance of bottled waters on retail sale in the Republic of Ireland with the microbiological criteria laid down in *European Communities (Natural mineral waters, spring waters and other waters in bottles or containers) Regulations 2007* (S.I. No. 225 of 2007) as amended. The three types of bottled waters defined in the legislation were sampled, i.e. natural mineral water (NMW), spring water (SW) and other waters (OW).

A total of 973 samples taken by environmental health officers (EHOs) were analysed by the official food microbiology laboratories (OFMLs) in the Health Service Executive (HSE) for one or more of the survey parameters. 11 samples did not meet the sample criteria and were excluded from analysis. This report therefore presents the results for 962 samples. OW and NMW each accounted for approximately a third of samples (34.8% and 34.3% respectively) while SW accounted for 19.6%. 108 samples (11.2%) could not be categorised into sample type based on the information provided.

EHOs investigated non-conformances and took appropriate follow-up action with manufacturers and distributors regarding individual unsatisfactory results. Typical follow-up actions included product withdrawals by food business operators (FBOs), investigations by EHOs at bottling plants within the Republic of Ireland, re-sampling and verification of results by EHOs and FBOs as well as bottling plant voluntary closure and corrective action by FBOs.

The main findings were as follows:

- 1.0% (10/960) of samples contained *Escherichia coli*, the primary indicator of faecal contamination in water and 0.2% (2/955) of samples contained enterococci, also an indicator of faecal contamination. The numbers of these organisms in samples were low. Nevertheless, these samples were considered to be unfit for human consumption.
- 0.8% (8/955) of samples contained *Pseudomonas aeruginosa*. The organism is commonly found in water, soil and faeces and is capable of growth in low nutrient aquatic environments^(14, 16). It is an opportunistic pathogen that rarely causes illness in healthy individuals but may be associated with hospital acquired infections^(14, 16, 18).
- 6.3% (60/960) of samples contained coliforms whose presence is an indication of possible faecal contamination and warrants further investigation. The presence of coliforms raises concerns regarding the standards of hygiene of the source or in the bottling process.
- 7.2% (69/952) of samples analysed for the four parameters (i.e. coliforms, *E. coli*, enterococci and *P. aeruginosa*) were found to not meet legal standards or were unsatisfactory compared to guideline criteria. The breakdown by sample type was as follows: 9.0% (17/188) of SW samples and 5.5% (18/326) of NMW samples did not meet legal microbiological standards whilst 8.4% (28/332) of OW samples

and 5.7% (6/106) of uncategorised samples were unsatisfactory compared to guideline criteria.

- A comparison of the microbiological results for the three types of bottled water (NMW, SW and OW) found that they were similar* for coliforms. Statistical analysis of difference was not appropriate for the other three parameters reported due to the low numbers of positive samples.

* No statistical difference (alpha = 0.05 significance level)

1. Introduction

The concept of bottling and selling water from Ireland was introduced in the early 1980s. Since then the bottled water industry has thrived and numerous brands (both domestically produced and imported) are now firmly established on the Irish market. In Ireland we now consume 193 million litres of bottled water per year ⁽¹⁾.

The success of the bottled water market is a global phenomenon. It has been attributed to a number of factors including consumer concerns about tap water (bottled water is often perceived to be safer and healthier than tap water) and a trend towards healthier living. The latter has probably been the biggest driving force as consumers seek a healthy and safe alternative to soft drinks and recognise the need to stay hydrated (the drinking water needs for individuals vary but it is estimated to be 2 litres per day for a 60 kg person and 1 litre per day for a 10 kg child ⁽²⁾ ^(1,3)).

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 ^{(4)∅}). They are:

1. Natural mineral water (NMW),
2. Spring water (SW) and
3. Other waters (OW)

which are intended for human consumption supplied in a bottle or container, other than waters which are medicinal products.

The National Standards Authority of Ireland (NSAI) describe NMW & 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 ⁽⁵⁾. Further, NMW is clearly distinguished from other drinking water by its nature (i.e. its content of minerals and trace elements), by its original state, it is bottled at source and is recognised as a NMW by the responsible authority ⁽⁵⁾. In Ireland this responsibility to recognise NMW lies with the NSAI. Water which is intended for human consumption and is placed on the market in either bottles or containers but is neither a NMW or a SW, falls into the category of OW. Further information on the differences between these three types of bottled water is provided in Appendix A.

[∅] S.I. No. 225 of 2007 (as amended) transposes two EC Directives:

- [Council Directive 80/777/EEC](#) (OJ L229, p1, 30/08/1980) on the approximation of the laws of the member states relating to the exploitation and marketing of natural mineral waters as amended by Council Directive 96/70/EC and Commission Directive 2003/40/EC
- [Council Directive 98/83/EC](#) (OJ L330, p32, 5/12/1998) of 3 November 1998 on the quality of water intended for human consumption.

The microbiological safety of bottled water is of paramount importance considering it is readily consumed by all sectors of the population. Its safety 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 procedures. To date no outbreak of human disease linked to bottled water has been reported in Ireland. Internationally, bottled water has been implicated as the vehicle in the transmission of cholera in a very well documented outbreak in Portugal in the 1970s in which the spring became contaminated by sewage infiltration through a limestone stratum ^(6,7). Bottled water has also been implicated as the vehicle of transmission in outbreaks of typhoid ⁽⁸⁾ and “travellers disease” ⁽⁹⁾ and more recently in an outbreak of *Salmonella* Kottbus in Gran Canaria where carrier pigeons were thought to have had access to a reservoir for the bottling plant ⁽¹⁰⁾.

Microbiological standards [♦] for bottled water are laid down in legislation and they are different for the different types of water ⁽⁴⁾. The criteria for NMW and SW are summarised in Appendix B (Table B1). The total colony count of these waters should be measured within 12 hours of bottling and should not exceed 100/ml or 20/ml depending on the incubation temperature and time. At source, and during marketing, the water must also be free from parasites, pathogenic organisms, *Escherichia coli*, coliforms, faecal streptococci, sporulated sulphite-reducing anaerobes and *Pseudomonas aeruginosa*. During marketing, colony counts are allowed to increase but ‘no more than that resulting from the normal increase in the bacteria content which the water had at source’⁽⁴⁾. The indigenous bacteria in bottled water have been shown to increase after bottling and then gradually decrease ^(11, 12). Each individual species will decrease in number once its particular food supply has been exhausted and as it dies and decays its constituents provide a food supply for other species.

The microbiological status of NMW and SW depends on the protected nature of the source and the hygiene of the bottling plant, as these waters can not receive treatments likely to change the viable colony count of the water.

OW must comply with specific microbiological standards at the point of bottling. These criteria are summarised in Appendix B (Table B2) and also apply to spring waters at the point of bottling. No specific criteria apply to OW during marketing; however, they must not be placed on the market unless they are wholesome and clean, i.e. they are free from any micro-organisms and parasites and from any substances which, in numbers or concentrations, constitute a potential danger to human health. In this report microbiological standards for OW at the point of bottling were applied as ‘guideline criteria’ for the assessment of the microbiological quality of OW on the market. Unlike NMW and SW, disinfection treatments may be applied to OW in order to remove pathogenic microorganisms however, this does not mean that contaminated water can be used as a source for OW.

[♦] A microbiological standard is a microbiological criterion contained in law where compliance is mandatory

Food safety is not guaranteed by microbiological testing alone; rather, it is ensured by a preventative approach such as the implementation of a food safety management system based on the principles of Hazard Analysis and Critical Control Point (HACCP) and good hygiene practice (GHP). Both of these are legal requirements. Article 5 of Regulation 852/2004 on Hygiene of Foodstuffs ⁽¹³⁾ requires food business operators (FBOs) to put in place, implement and maintain a permanent procedure or procedures based on the principles of HACCP; while, Article 4 requires FBOs to comply with general and specific hygiene requirements (i.e. GHP).

A number of product standards are available to packagers of bottled water. At national level, the National Standards Authority of Ireland (NSAI) has produced a standard (I.S. 432:2005 Packaged Water) for packagers of Irish ground water ⁽⁵⁾. This standard contains specific requirements for certified waters (compliance is mandatory) and recommendations (compliance is voluntary). At international level, a standard relevant to bottled water has been published by the World Health Organisation ⁽¹⁴⁾ and a number of standards have been published by the Codex Alimentarius Commission ⁽¹⁵⁾:

- Codex Standard for Natural Mineral Waters (108 - 1981 Rev 1 1997)
- Recommended International Code of Hygienic Practice for the Collecting, Processing and Marketing of Natural Mineral Waters (CAC/RCP 33-1985)
- General Standard for Bottled/Packaged Drinking Waters (other than Natural Mineral Waters) (227-2001)
- Code of Hygienic Practice for Bottled/Packaged Drinking Waters (other than Natural Mineral Waters) (CAC/RCP 48-2001)

2. Specific Objectives

The aim of this study was to investigate compliance of bottled waters on retail sale in the Republic of Ireland with the microbiological criteria laid down in S.I. No. 225 of 2007.

3. Method

3.1 Sample source

Environmental health officers (EHOs) were requested to sample from the following types of premises:

- Supermarkets
- Corner shops
- Hotels
- Restaurants
- Pubs
- Vending machines
- Ethnic stores

3.2 Sample description

The three types of bottled waters were sampled:

- i) Natural mineral waters
- ii) Spring waters
- iii) Other waters

Samples included:

- Water supplied in a bottle (or container)
- Effervescent and still water

The following were specifically excluded from this survey:

- Flavoured water
- Unbottled water (e.g. tap water, water from cooling dispensers etc)
- Waters (bottled/unbottled) which are medicinal products

3.3 Sample collection

Sampling was undertaken by EHOs from the Health Service Executive (HSE) during September, October, November and December 2007. The samples, which were taken at random across the country, cover a wide range of waters, but given the large number of products available from both domestic and imported producers, may not have covered all products on the market. Multiple sampling of major brands was unavoidable, however, to minimise this problem, EHOs were requested to:

- submit no more than one sample of ‘natural mineral water’, one sample of ‘spring water’ and one sample of ‘other water’ per brand/trade name from each premises
- sample local/own brands where available.

The minimum sample size was 1 litre. Where a sample consisted of more than one bottle (e.g. 2x750 ml bottle, 2x500 ml, 4x250 ml bottles, etc.), the bottles were from the same production batch. All samples were transported to the laboratory under appropriate conditions (i.e. in a cool box). EHOs completed sections 1, 2 & 3 of the questionnaire provided (Appendix C) at the time of sampling.

3.4 Sample analysis

Samples were submitted to the HSE official food microbiology laboratories (OFMLs) for analysis. Samples were analysed for the following parameters using the analytical reference methods which are specified in S.I. No. 225 of 2007 (Table 1).

- Total coliforms
- *E. coli*
- Intestinal enterococci
- *P. aeruginosa*
- Aerobic colony count (at 22°C and 37°C)

Table 1: Analytical reference methods (S.I. No. 225 of 2007)

Parameter	Method*
Coliform bacteria and <i>E. coli</i>	ISO 9308-1
Enterococci	ISO 7899-2
<i>P. aeruginosa</i>	prEN ISO 12780
Enumeration of culturable microorganisms — Colony count 22°C	prEN ISO 6222
Enumeration of culturable microorganisms — Colony count 37°C	prEN ISO 6222

***Note:** Member states are permitted to use alternative methods, providing the provisions of Article 7(5) of Council Directive 98/83/EC are met, i.e. *‘Methods other than those specified may be used, providing it can be demonstrated that the results obtained are at least as reliable as those produced by the methods specified. Member States which have recourse to alternative methods shall provide the Commission with all relevant information concerning such methods and their equivalence’.*

3.5 Reporting of Results

Laboratory reports were forwarded to EHOs and the Food Safety Authority of Ireland (FSAI) using the normal reporting channels. Laboratory reports were requested to be forwarded to the FSAI within one month of the survey completion date. Laboratory reports of follow-up samples were not included in this report as this type of sampling is not random and hence would bias the original data set. Results of samples that did not meet the sampling criteria were also excluded.

3.6 Questionnaire data

Upon receipt of the laboratory results, EHOs completed the questionnaire (Appendix C) and this was requested to be sent to FSAI within six weeks of the survey completion date. Questionnaires received after this period were excluded from the survey report.

3.7 Statistical analysis

Chi square and Fisher’s Exact Test analysis was performed using SPSS version 14.0. (alpha = 0.05 significance level).

4. Results and Discussion

A total of 973 samples taken by EHOs were analysed by the OFMLs for one or more of the survey parameters. Details of samples taken by HSE regions and laboratories are provided in Appendix D. 11 samples did not meet the sample criteria therefore this report presents the results for 962 samples.

The type of premises from which samples were taken is presented in Figure 1. The majority of samples (90.5%) were taken at retail shops.

Figure 1: Premises categories sampled (n=962)

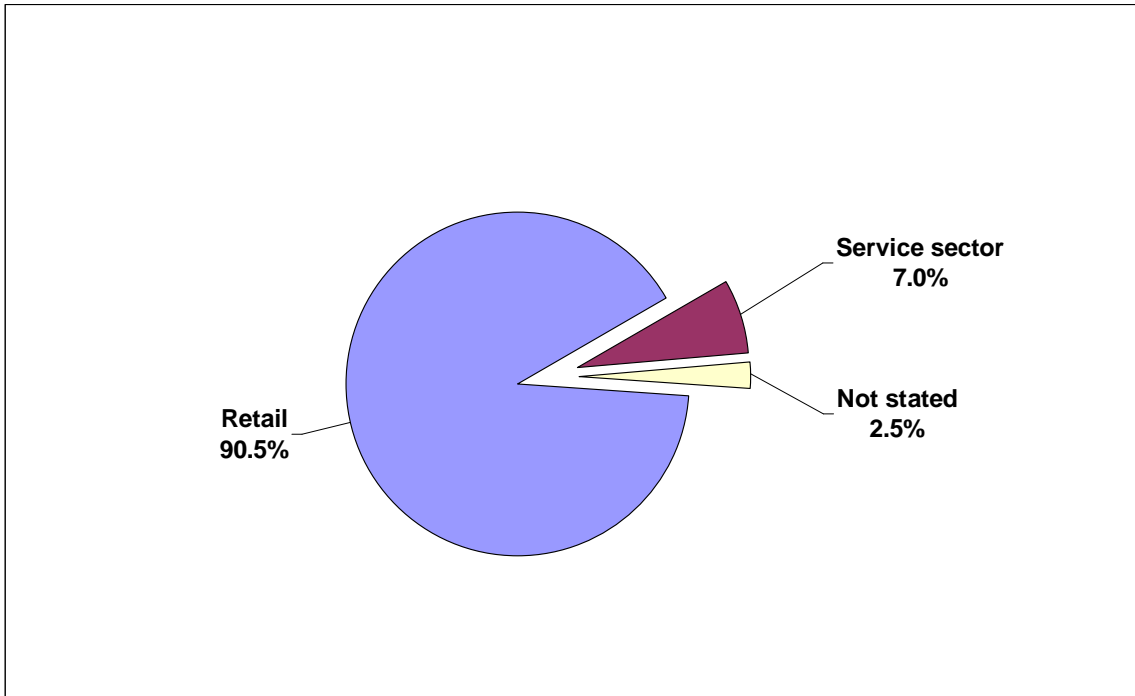
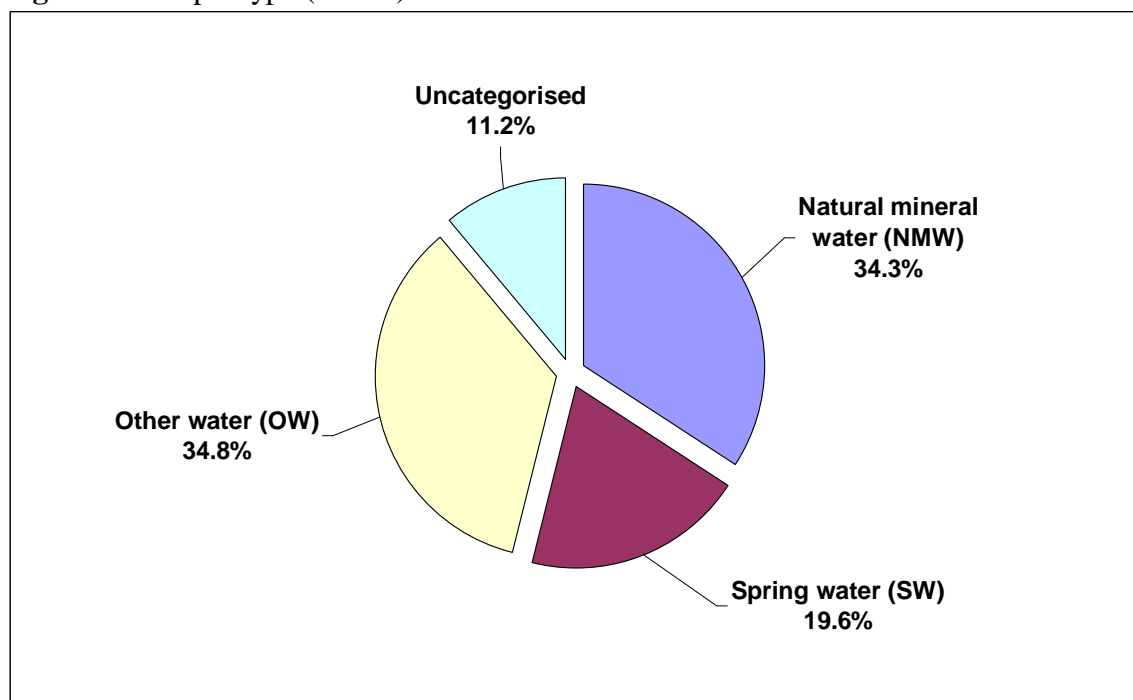


Figure 2 presents the breakdown of samples by sample type (i.e. natural mineral water (NMW), spring water (SW) and other water (OW)). OW and NMW each accounted for approximately a third of samples (34.8% and 34.3% respectively) while SW accounted for 19.6%. 108 samples (11.2%) could not be categorised into sample type based on the information provided.

Figure 2: Sample type (n=962)

4.1 Microbiological Results

4.1.1 Results for all bottled waters

Samples were analysed for one or more of the following microbiological parameters: total coliforms, *E. coli*, intestinal enterococci and *P. aeruginosa*. Details of the number of samples tested for each parameter and the corresponding results are provided in Table 2. The coliform indicator was the parameter for which the highest percentage of samples was reported to be positive (i.e. 6.3% of samples tested). Coliform bacteria belong to the family Enterobacteriaceae. Typical genera belonging to the coliform group found in water include *Citrobacter*, *Enterobacter*, *Escherichia*, *Hafnia*, *Klebsiella*, *Serratia* and *Yersinia*⁽¹⁶⁾. These organisms are not exclusively of faecal origin and therefore failure of the total coliform test is considered an indication of possible faecal contamination and in the case of tap water it could also indicate a failure of chlorination^(14, 16, 17). The presence of coliforms in bottled water raises concerns regarding the standards of hygiene of the water source or in the bottling process.

Table 2: Presence of coliforms, *E. coli*, enterococci and *P. aeruginosa* in all samples (n=962)

Microbiological Parameter	No. of samples tested	Samples Not detected/250ml		Samples Detected /250ml	
		Number	%	Number	%
Coliforms	960	900	93.7	60	6.3
<i>E. coli</i>	960	950	99.0	10	1.0
Enterococci	955	953	99.8	2	0.2
<i>P. aeruginosa</i>	955	947	99.2	8	0.8

952 samples were examined for all four parameters. 7.2% (69/952) of these samples were found to be positive for one or more of the four parameters (Table 3). 10 of the 60 samples which were positive for coliforms were also positive for *E. coli*. Additionally, one of these 10 contained enterococci.

E. coli is a coliform bacterium which occurs in the faeces of all mammals and is regarded as the primary indicator of faecal contamination in water ^(14, 16, 17). Enterococci include a number of species which occur in faeces of humans and warm blooded animals ⁽¹⁶⁾. They are generally more resistant than *E. coli* and coliforms to environmental stress and generally survive longer in water environments ^(14, 16). Enterococci are also a group of organisms that indicate faecal contamination of water ⁽¹⁴⁾.

Eight samples (0.8%) were positive for *P. aeruginosa*. The organism is commonly found in water, soil and faeces and is capable of growth in low nutrient aquatic environments ^(14, 16). It is an opportunistic pathogen that rarely causes illness in healthy individuals but may be associated with hospital acquired infections ^(14, 16, 18). A review of several reports of hospital infections attributable to *P. aeruginosa* in hospital water supplies has also been conducted ⁽²²⁾. As the concentration of *P. aeruginosa* in bottled water in this study was low (6 to 100 cfu/250 ml) the risk of infection even for vulnerable patients was likely to be low.

Table 3: All samples which were analysed for all four parameters (n=952)

Microbiological Parameter	No. of samples positive	% of samples positive
Coliforms only	50	5.3
Enterococci only	1	0.1
<i>P. aeruginosa</i> only	8	0.8
<i>E. coli</i> & coliforms	9	0.9
<i>E. coli</i> & coliforms & enterococci	1	0.1
Positive for one or more parameter	69	7.2

Table 4 presents the results by colony forming units (cfu) detected per 250ml. For each parameter the majority of samples had counts <10 cfu/250 ml. The one sample which was positive for three of the parameters had the following counts: *E. coli* (2 cfu/250 ml), coliforms (30 cfu/250 ml) and enterococci (2 cfu/250 ml). The highest coliform count reported was 20,000 cfu/250 ml. This sample was not positive for any of the other three parameters. It should be noted that the numbers of *E. coli* and enterococci in samples were low. Nevertheless, these samples were classified as unsafe[†] because they were unfit for human consumption.

[†] Article 14 (2) of Regulation (EC) No 178/2002 on general food law states that: "Food shall be deemed to be unsafe if it is considered to be (a) injurious to health (b) unfit for human consumption"

Table 4: Colony forming units (cfu) detected in 250 ml for coliforms, *E. coli*, enterococci and *P. aeruginosa* in all samples (n=962)

Parameter	No. of samples tested	Colony forming units (cfu) per 250ml				
		Not detected	1 to <10	10 to <20	20 to <100	≥100
Coliforms	960	900 (93.8%)	31 (3.2%)	12 (1.3%)	14 (1.5%)	3 (0.3%)
<i>E. coli</i>	960	950 (99.0%)	9 (0.9%)	1 (0.1%)	0 (0%)	0 (0%)
Enterococci	955	953 (99.8%)	2 (0.2%)	0 (0%)	0 (0%)	0 (0%)
<i>P. aeruginosa</i>	955	947 (99.2%)	1 (0.1%)	2 (0.2%)	4 (0.4%)	1 (0.1%)

4.1.2 Results by sample type

The presence of coliforms, *E. coli*, enterococci and *P. aeruginosa* for each of the different types of bottled waters sampled (natural mineral water (NMW), spring water (SW), other water (OW) and those which could not be categorised) is presented in Table 5.

Table 5: Presence of coliforms *E. coli*, enterococci and *P. aeruginosa* in NMW, SW, OW and uncategorised waters.

Parameter	NMW (n=330)		SW (n=189)		OW (n=335)		Uncategorised (n=108)	
	No. of samples tested	No. (%) of samples positive	No of samples tested	No. (%) of samples positive	No. of samples tested	No. (%) of samples positive	No. of samples tested	No. (%) of samples positive
Coliforms	329	15 (4.6)	189	13 (6.9)	334	28 (8.4)	108	4 (3.7)
<i>E. coli</i>	329	0 (0.0)	189	2 (1.1)	334	7 (2.1)	108	1 (0.9)
Enterococci	327	0 (0.0)	188	1 (0.5)	334	0 (0.0)	106	1 (0.9)
<i>P. aeruginosa</i>	328	3 (0.9)	188	3 (1.6)	333	0 (0.0)	106	2 (1.9)

A comparison of the microbiological results for the three types of bottled water (NMW, SW and OW) found that they were similar[‡] for coliforms. Statistical analysis of difference was not appropriate for the other three parameters reported due to the low numbers of positive samples.

A. Natural Mineral Water (NMW)

Microbiological standards for coliforms, *E. coli*, enterococci and *P. aeruginosa* are laid down in legislation for natural mineral water (NMW) when placed on the market[§]. Table 5 presents the results for the 330 NMW samples analysed. 326 samples were analysed for all four parameters. 5.5% (18/326) were found to be unsatisfactory according to the legal criteria for one or more microbiological parameter. 15 (4.6%) of these samples were unsatisfactory for coliforms and three (0.9%) different samples were unsatisfactory for *P. aeruginosa*.

B. Spring Water (SW)

Microbiological standards for coliforms, *E. coli*, enterococci and *P. aeruginosa* are laid down in legislation for Spring Water (SW) when placed on the market^{**}. Table 5 presents the results for the 189 SW samples analysed. 188 samples were analysed for all four parameters (Table 6). 9.0% (17/188) were found to be unsatisfactory according to the legal criteria for one or more microbiological parameter. 13 of these samples were unsatisfactory for coliforms and two of these 13 were also found to be unsatisfactory for *E. coli*. Three samples were unsatisfactory for *P. aeruginosa* and one sample was unsatisfactory for enterococci.

Table 6: SW samples which were analysed for all four parameters (n=188)

Microbiological Parameter	No. of samples positive	% of samples positive
Coliforms only	11	5.9
Coliforms & <i>E. coli</i>	2	1.1
Enterococci only	1	0.5
<i>P. aeruginosa</i> only	3	1.6
Positive for one or more parameter	17	9.0

C. Other Water (OW)

Microbiological standards for coliforms, *E. coli*, enterococci and *P. aeruginosa* are laid down in legislation for Other Water (OW) at the point at which the water is placed in the bottles. As the samples in this survey were taken at retail level we used the point of bottling standards as 'guideline criteria' for the purposes of this survey. Table 5 presents

[‡] No statistical difference (alpha = 0.05 significance level)

[§] Placing on the market is defined in Article 3 of Regulation (EC) No 178/2002:

- 'placing on the market' means the holding of food or feed for the purpose of sale, including offering for sale or any other form of transfer, whether free of charge or not, and the sale, distribution, and other forms of transfer themselves;

the results for the 335 OW samples analysed. 332 samples were analysed for all four parameters. 8.4% (28/332) were found to be unsatisfactory according to the guideline criteria for one or more microbiological parameter. Seven (2.1%) of these samples were unsatisfactory for both *E. coli* and coliforms. 21 (6.3%) samples were unsatisfactory for coliforms only.

D. Uncategorised samples

108 samples could not be categorised into sample type based on the information provided and were therefore evaluated using the 'guideline criteria' previously mentioned. 106 of these were analysed for all four parameters. 5.7% (6/106) were found to be unsatisfactory according to the guideline criteria for one or more microbiological parameter (Table 7). One of these samples was found to be unsatisfactory for three parameters (i.e. *E. coli*, coliforms and enterococci). Three samples were unsatisfactory for coliforms only and two samples were unsatisfactory for *P. aeruginosa* only.

Table 7: Uncategorised samples which were analysed for all four parameters (n=106)

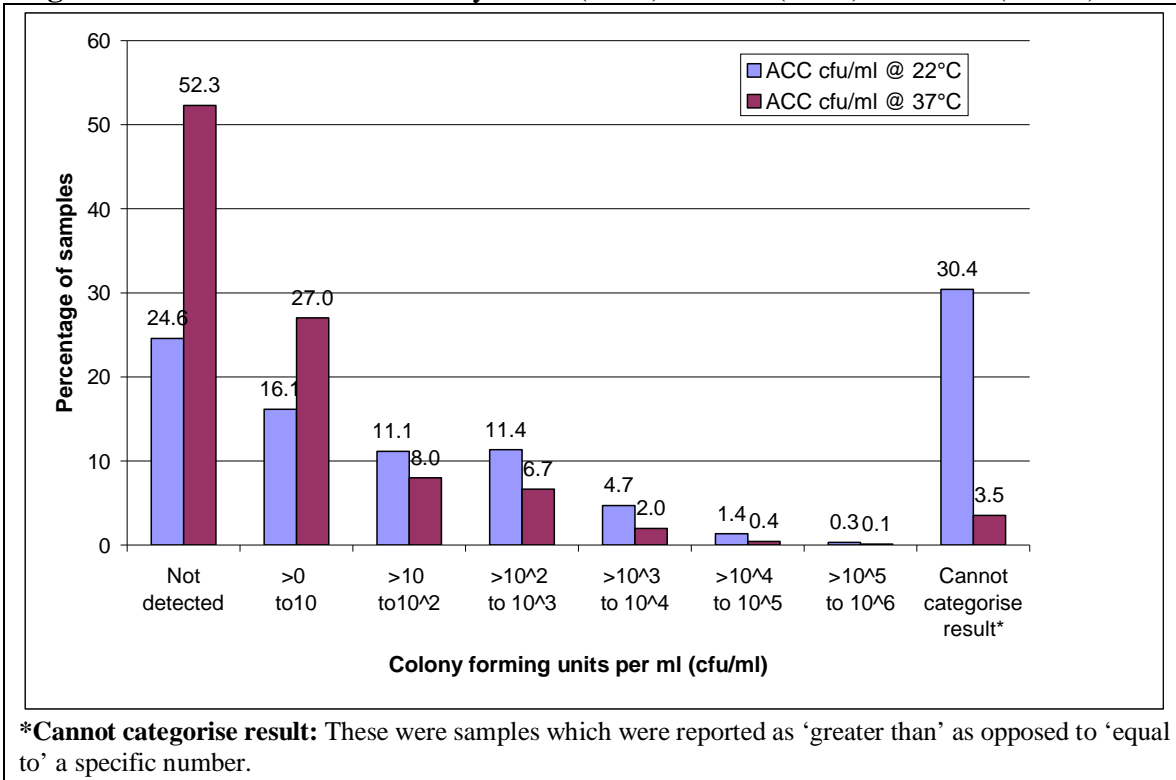
Microbiological Parameter	No. of samples positive	% of samples positive
Coliforms only	3	2.8
Coliforms & <i>E. coli</i> & enterococci	1	0.9
<i>P. aeruginosa</i> only	2	1.9
Positive for one or more parameter	6	5.7

4.2 Microbiological Results for Aerobic Colony Counts (ACC)

The indigenous bacteria in bottled water have been shown to increase after bottling and then gradually decrease^(11, 12). Each individual species will decrease in number once its particular food supply has been exhausted and as it dies and decays its constituents provide a food supply for other species. Consequently sampling of bottled water at any point in time provides a snapshot with the result depending on the initial bacterial load in the source water and the time lapsed since the water was bottled.

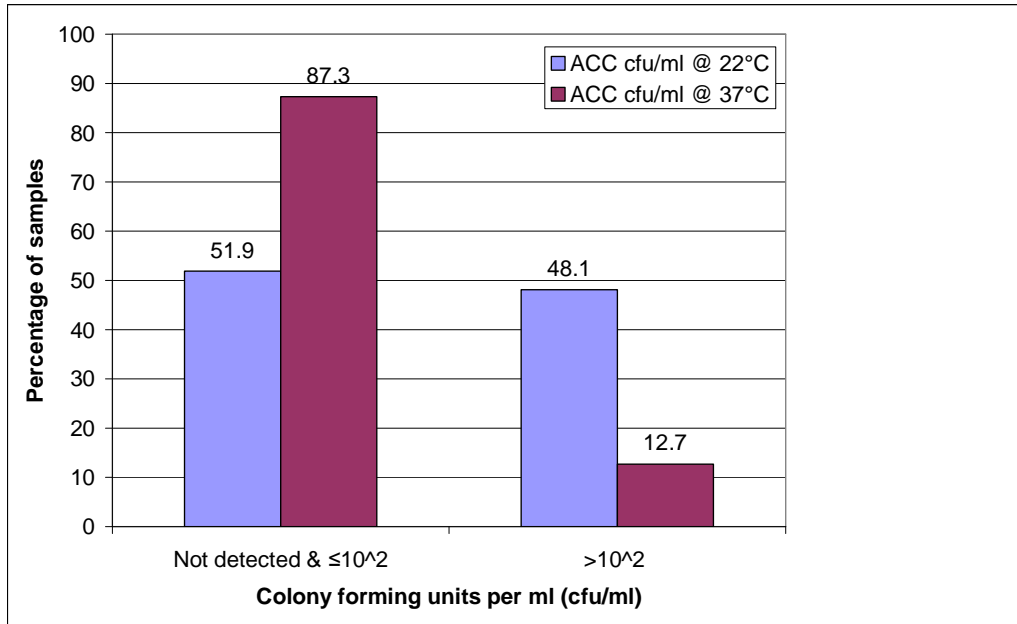
In this study samples were analysed for aerobic colony count (ACC) at 22°C (n=960) and 37°C (n=962), the results are presented in Figure 3. Approximately half of the samples analysed at 37°C (52.3%) and a quarter of the samples analysed at 22°C (24.6%) had no colonies detected.

Figure 3: Results for aerobic colony count (ACC) at 22°C (=960) and 37°C (n=962)



30.4% of the results for samples analysed at 22°C and 3.5% for those analysed at 37°C were reported as 'greater than' as opposed to 'equal to' a specific number and were therefore labelled as 'Cannot categorise result' in Figure 3. As all of these samples were >100 cfu/ml we can report that overall 48.1% of all samples analysed at 22°C and 12.7% of all those analysed at 37°C were >100 cfu/ml (Figure 4).

Figure 4: Results of samples analysed for aerobic colony count (ACC) at 22°C (=960) and 37°C (n=962)



The bottled water legislation sets total colony count (ACC) standards for NMW and SW sampled within 12 hours of bottling: requiring that the count at 22°C does not exceed 100 cfu/ml and the count at 37°C does not exceed 20 cfu/ml. While on the market however, it requires that the count should only be that which would result from the normal increase in the count which the sample had at source. Therefore the ACC results in this study cannot be used to assess compliance with the legislation as the initial count is unknown. The results provide a snapshot of the microbial load and are dependent on the initial bacterial count in the source water for each bottle and the time lapsed since the water was bottled. The ACC results for NMW and SW are presented in Appendix E

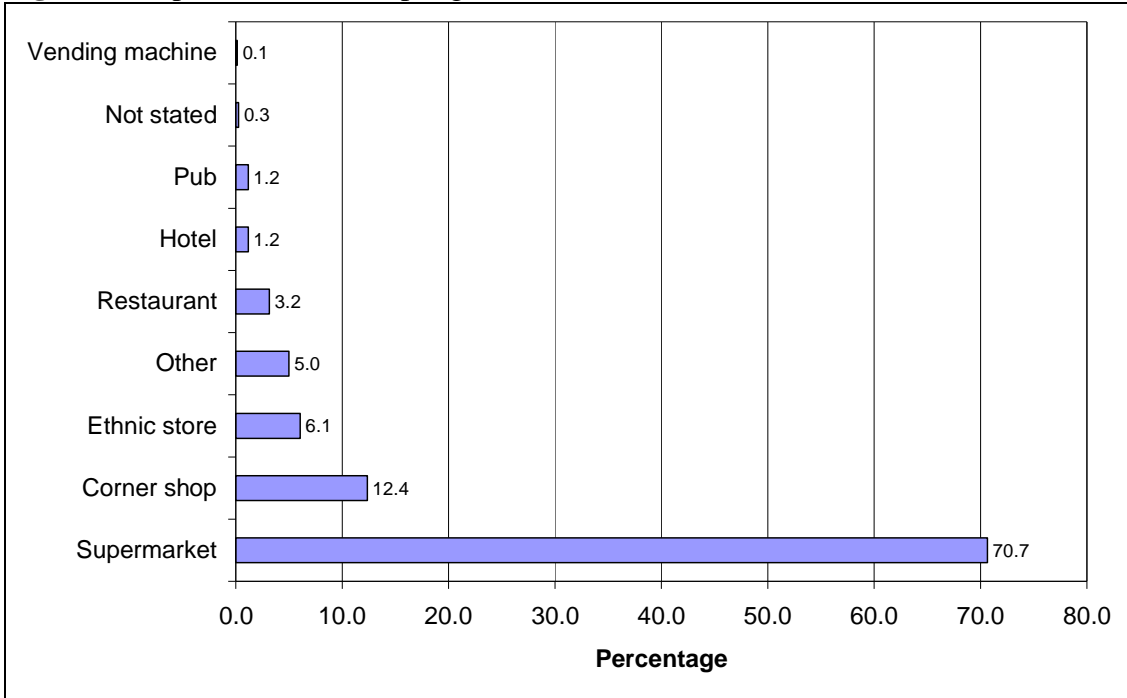
The bottled water legislation sets total colony count (ACC) standards for OW at the time of bottling: requiring that the count at 22°C does not exceed 100 cfu/ml and the count at 37°C does not exceed 20 cfu/ml. Unlike NMW and SW criteria are not set for OW while on the market. The ACC results for OW and the uncategorised samples are presented in Appendix E.

4.3 Analysis of Questionnaire Data

Questionnaires were returned for 765 samples of the 973 samples (response rate 78.6%). Five of the samples with questionnaires returned were excluded as they did not meet the sample criteria. Therefore the results of 760 samples with valid questionnaires are presented below.

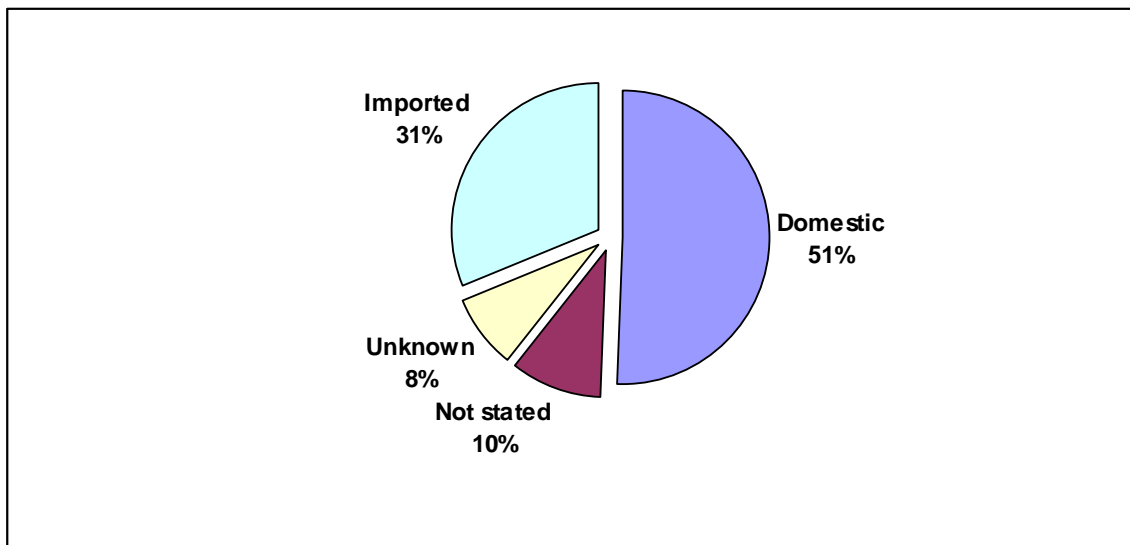
Regarding the site of sampling, the majority of samples were reported to have been taken from supermarkets (Figure 5).

Figure 5: Reported site of sampling (n=760)



Approximately half the samples for which valid questionnaires were available were classified as domestic with a third being classified as imported (Figure 6).

Figure 6: Reported origin of samples (domestic or imported; n=760)



8.9% of the samples reported to be of domestic origin were found to contain coliforms compared to 0.8% of samples reported to be imported (Table 8). This finding was

statistically significant ($p=0.00003$). However, the difference in these coliform results maybe due to bias in sampling which in the case of domestic samples would have included multiple positive samples from the same bottled water source. Therefore definitive conclusions concerning the difference in coliform status between imported and domestic bottled water are not possible. The results for *E. coli*, enterococci and *P. aeruginosa* (Table 8) were found to be similar irrespective of reported origin **.

Table 8: Results of samples reported to be of either domestic or imported origin

Parameter	Domestic		Imported	
	No. of samples with reported origin ^a	No. (%) of samples positive	No. of samples with reported origin ^a	No. (%) of samples positive
Coliforms	384	34 (8.9%)	237	2 (0.8%)
<i>E. coli</i>	384	6 (1.6%)	237	0 (0.0%)
Enterococci	383	0 (0.0%)	237	1 (0.4%)
<i>P. aeruginosa</i>	383	2 (0.5%)	236	5 (2.1%)

^a Not all samples in the study had a questionnaire associated with them and some questionnaires did not provide information on origin.

Figure 7 presents the breakdown of samples by whether they were reported to be effervescent or still. 20% of samples were reported to be effervescent (i.e. naturally carbonated, carbonated or fortified with gas). It has been observed that in carbonated waters the survival rate of some bacteria reduce by 25-50% ⁽¹⁷⁾. In this study the difference in coliform results between still and effervescent samples was found to be statistically significant ($p=0.004$) with 7.7% of still samples containing coliforms compared to 1.3% of effervescent samples (Table 9). The results for the other parameters were similar between both water types^{††}.

** No statistical difference ($\alpha=0.05$ significance level)

Figure 7: Samples which were effervescent or still (n=760)

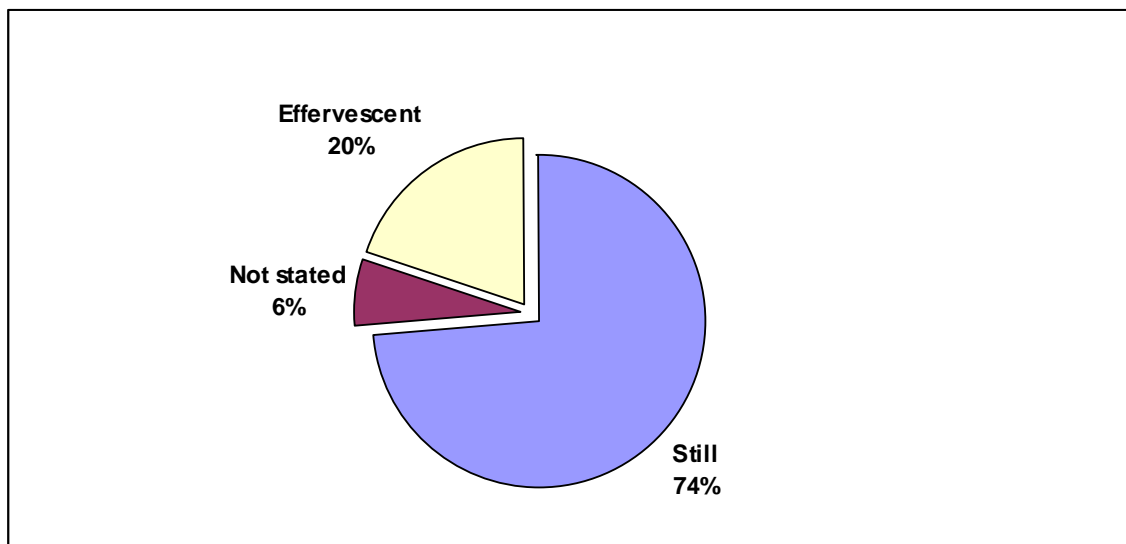


Table 9: Results of still and effervescent samples

Parameter	Still		Effervescent	
	No. of samples tested	No. (%) of samples positive	No. of samples tested	No. (%) of samples positive
Coliforms	559	43 (7.7%)	152	2 (1.3%)
<i>E. coli</i>	559	9 (1.6%)	152	0 (0.0%)
Enterococci	557	1 (0.2%)	152	0 (0.0%)
<i>P. aeruginosa</i>	556	6 (1.1%)	152	1 (0.7%)

Table 10 reproduces results from the most recent Environmental Protection Agency (EPA) report on Irish drinking water ⁽¹⁹⁾. While a direct statistical comparison between domestically produced bottled water samples and Irish tap water supplies is not appropriate, due to the differences in how the water is treated and the data is presented (results for supplies rather than samples), it is interesting to note that in 2006, 8.3% of public supplies were found to contain *E. coli*/100 ml. The public supply serves 81.8% of the Irish population.

Table 10: Irish tap water supplies with exceedances in 2006 (Derived from EPA report ⁽¹⁹⁾)

Parameter	% of water supplies with exceedances i.e. Presence/100 ml (No. of supplies with exceedances / No. of supplies monitored)		
	Public supplies	Public group schemes	Private group schemes
<i>E. coli</i>	8.3% (77/931)	1.8% (12/666)	35.8% (246/688)
Enterococci	3.7% (26/712)	1.3% (2/153)	14.3% (30/210)
Coliforms	31.6% (294/930)	11.2% (74/663)	54.8% (377/688)

A survey in Greece of 1,527 samples of still bottled water sampled between 1995 and 2003 ⁽²⁰⁾ found the following contamination levels: *E. coli* (1%), coliforms (11%), enterococci (1.2%) and *P. aeruginosa* (5.9%). Direct comparisons with the present study are not valid due to differences in sampling and analysis methods.

5. Conclusions

In this study 7.2% (69/952) of samples analysed for the four parameters (i.e. coliforms, *E. coli*, enterococci and *P. aeruginosa*) were found to not meet legal standards or were unsatisfactory compared to guideline criteria. The breakdown by sample type was as follows: 9.0% (17/188) of SW samples and 5.5% (18/326) of NMW samples did not meet legal microbiological standards whilst 8.4% (28/332) of OW samples and 5.7% (6/106) of uncategorised samples were unsatisfactory compared to guideline criteria.

EHOs investigated non-conformances and took appropriate follow-up action with manufacturers and distributors regarding individual unsatisfactory results. Typical follow-up actions included product withdrawals by FBOs, investigations by EHOs at bottling plants within Republic of Ireland, re-sampling and verification of results by EHOs and FBOs as well as bottling plant voluntary closure and corrective action by FBOs.

1.0% (10/960) of samples contained *E. coli*, the primary indicator of faecal contamination in water and 0.2% (2/955) of samples contained enterococci, also an indicator of faecal contamination. The numbers of these organisms in samples were low. Nevertheless, these samples were considered to be unfit for human consumption^{‡‡}.

^{‡‡} Legally, such products are regarded as “unsafe”. Article 14 (2) of Regulation (EC) No 178/2002 on general food law states that: “Food shall be deemed to be unsafe if it is considered to be (a) injurious to health (b) unfit for human consumption”

6.3% (60/960) of samples contained coliforms whose presence is an indication of possible faecal contamination and warrants further investigation. The presence of coliforms raises concerns regarding the standards of hygiene of the source or in the bottling process. Microbiological results for the three types of water (NMW, SW, OW) were similar^{§§} for coliforms and statistical comparison was not possible for other parameters due to the low number of positive samples.

0.8% (8/955) of samples contained *P. aeruginosa* (i.e. (0.9% (3/328) of NMW and 1.6% (3/188) of SW samples). It is an opportunistic pathogen that rarely causes illness in healthy individuals but may be associated with hospital acquired infections ^(14, 16, 18).

The recommendations from this survey are as follows:

1. Irish bottled water manufacturers should review their good manufacturing practices and food safety management systems to ensure that they produce water which meets the requirements of S.I. No. 225 of 2007.
2. Manufacturers in conjunction with NSAI should review and, if necessary, revise, the National Standard for packaged water (I.S. 432:2005).
3. During the proposed revision of FSAI Code of Practice No. 1, the risk categorisation of bottled water plants should be re-examined.
4. Retail level sampling of bottled water should periodically be included in routine microbiological surveillance work under official controls.
5. Manufacturers must take reasonable measures to ensure that bottled water complies with any applicable legislation. Bottled water failing legal microbiological standards should be removed from the market.

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^{§§} No statistical difference (alpha=0.05 significance level)

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7. Appendices

APPENDIX A

Summary of principal legislation pertaining to bottled water (*list not exhaustive*)

1) Product specific legislation

Natural Mineral Waters:

- [Council Directive 80/777/EEC](#) (OJ L229, p1, 30/08/1980) on the approximation of the laws of the member states relating to the exploitation and marketing of natural mineral waters as amended by Council Directive 96/70/EC and Commission Directive 2003/40/EC.

Spring Waters:

- [Council Directive 80/777/EEC](#) (OJ L229, p1, 30/08/1980) on the approximation of the laws of the member states relating to the exploitation and marketing of natural mineral waters as amended by Council Directive 96/70/EC and Commission Directive 2003/40/EC.
- [Council Directive 98/83/EC](#) (OJ L330, pr32, 5/12/1998) of 3 November 1998 on the quality of water intended for human consumption.

Other Bottled Waters:

- [Council Directive 98/83/EC](#) (OJ L330, p32, 5/12/1998) of 3 November 1998 on the quality of water intended for human consumption.

All of the above Directives have been transposed into national legislation by *S.I. No. 225 of 2007* European Communities (Natural Mineral Waters, Spring Waters and Other Waters in Bottles or Containers) Regulations 2007 as amended by S.I. No. 686 of 2007.

2) Hygiene requirements

- Regulation (EC) No 852/2004 of the European parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs.

This Regulation has been transposed into national legislation by S.I. No. 369 of 2006, European Communities (Hygiene of Foodstuffs) Regulations 2006.

3) General Food law

- [Regulation \(EC\) No 178/2002](#) laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety as amended.

This Regulation has been transposed into national legislation by S.I. No. 747 of 2007, European Communities (General Food Law) Regulations 2007.

4) Other legislation

These include:

- Labelling legislation and
- Legislation for materials and articles intended to come in contact with food.

Table A1: A comparison of some legislative requirements for NMW, SW & OW*

Parameter	NMW	SW	OW
Recognition of water by the responsible authority of the member state	Yes [∞]	No	No
Must demonstrate source protection	Yes	Yes	No
Water must be bottled at source	Yes	Yes	No
Source of the water must be specified on the label	Yes	Yes	No
Only one brand of water comes from each source	Yes	Yes	No
Must have a constant composition	Yes [⊗]	No	No
Analytical composition of the water must be labelled	Yes	No [♥]	No
Water must be wholesome in its untreated state	Yes	Yes	No
Treatments permitted for the removal of unstable constituents/ undesirable substances	Yes [*]	Yes [*]	Yes
Treatments permitted for the removal of pathogenic micro-organisms	No [♦]	No [♦]	Yes
Safe to drink	Yes	Yes	Yes

* It is essential to consult the legislation for a complete interpretation as rules/exceptions exist for many of these parameters. This table was adapted from Dege, 2005⁽²¹⁾

[∞] To be recognised as a NMW, the water and source must be assessed. The source must be subject to a geological and hydrological survey and the water subject to microbiological, physical, chemical and physiochemical analyses. Clinical and pharmacological analyses must also be performed. In Ireland, the responsible authority is the National Standards Authority of Ireland (NSAI). Currently three NMWs are registered with the NSAI, i.e. Glenpatrick, Kerry Spring & Tipperary water. A list of natural NMWs recognised by Member States is available on the EU website (http://ec.europa.eu/food/food/labellingnutrition/water/mw_eulist_en.pdf).

[⊗] NMW must have a constant composition of certain mineral salts & trace elements.

[♥] Some spring waters may be labelled with the analytical composition; however, this is not a legal requirement.

^{*} The only treatments permitted are: filtration, decanting, pre-oxidation, treatment with ozone enriched air or any other treatment which does not alter the composition of the water as regards the essential constituents which give them their properties.

[♦] These waters can not be subjected to a disinfection treatment, the addition of bacteriostatic elements or any other treatment likely to change the viable count of the water.

APPENDIX B

Summary of Microbiological Criteria pertaining to bottled water

Table B1: Microbiological Criteria for Bottled Natural Mineral Water and Bottled Spring Water as specified in S.I. No. 225 of 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) 20/ml (37°C in 24 hours on agar-agar)	12 hour after bottling *
	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) & Regulation 11 (Paragraph 2d) of S.I. No. 225 of 2007.

Table B2: Microbiological Criteria for Bottled Spring Water and Other Bottled Waters as specified in S.I. No. 225 of 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 micro-organisms 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 & Schedule 5 of S.I. No. 225 of 2007.

APPENDIX C
Questionnaire 07NS3: Microbiological Safety and Quality of Bottled Water

1. General Information:

- * EHO Name: _____
- * EHO Sample Reference Number (i.e. EHO's own personal reference number for the sample) _____
- * Laboratory Reference Number (upon receipt of lab report) _____

2. Premises Information (See section 3 of Protocol):

- ✓ **Premises type:** Supermarket ; Corner shop ; Hotel ; Restaurant ; Pub ; Vending machine ; Ethnic store ;
 Other Please specify _____

3. Sample information:

- Is the product:** 'Natural mineral water' (NMW) or 'Spring water' or 'Other water'
If the product is a 'NMW' does it bear the words "water subjected to an authorised ozone-enriched air oxidation technique" Yes or No
If the product is a 'NMW' or a 'spring water' please provide details of the name of the spring: _____
- Is the water effervescent (i.e naturally carbonated, carbonated or fortified with gas):** Yes or No
Is the water imported: Yes or No or Unknown
Trade name: _____
Bottlers name & address: _____
Batch Number: _____
Best before date: _____
Storage conditions: Ambient or Refrigerated

4. Microbiological results (see section 8 of protocol):

	Satisfactory	Unsatisfactory
<i>E. coli</i>	<input type="checkbox"/>	<input type="checkbox"/>
<i>Faecal streptococci</i>	<input type="checkbox"/>	<input type="checkbox"/>
<i>Pseudomonas aeruginosa</i>	<input type="checkbox"/>	<input type="checkbox"/>
<i>Coliforms</i>	<input type="checkbox"/>	<input type="checkbox"/>
ACC (22°C)	<input type="checkbox"/>	<input type="checkbox"/>
ACC (37°C)	<input type="checkbox"/>	<input type="checkbox"/>

5. Follow-up action (see section 9 of protocol):

- (Follow-up action is only required for unsatisfactory results. Please tick as many boxes as necessary)
- None
 Repeat sample Lab. ref. no. of repeat sample : _____
 Other action Details: _____

APPENDIX D

Samples by Health Service Executive Region and by Laboratory

Table D1: Sample numbers per Health Service Executive (HSE) Region & Area

HSE Region	HSE Area	Number of samples submitted	No. of samples considered for this report
HSEDMLR	East Coast Area	47	47
	Midlands Area	84	84
	South Western Area	102*	98
HSEDNER	North Eastern Area	69	69
	Northern Area	80	80
HSESR	South Eastern Area	204	204
	Southern Area	134**	127
HSEWR	Mid-Western Area	94	94
	North Western Area	79	79
	Western Area	80	80
Total		973	962

* 102 samples submitted (four samples from distributors and transporters were excluded).

** 134 samples analysed (six samples from primary producers & one soft drink sample were excluded).

Table D2: Sample numbers per Official Food Microbiology Laboratory (OFML)

OFML	No. of samples analysed	No. of samples considered for this report
Cherry Orchard	201*	197
Cork	134**	127
Galway	80	80
Limerick	94	94
Sligo	79	79
SPD	181	181
Waterford	204	204
Total	973	962

* 201 samples analysed (four samples from distributors and transporters were excluded).

** 134 samples submitted (six samples from primary producers & one soft drink sample were excluded).

APPENDIX E

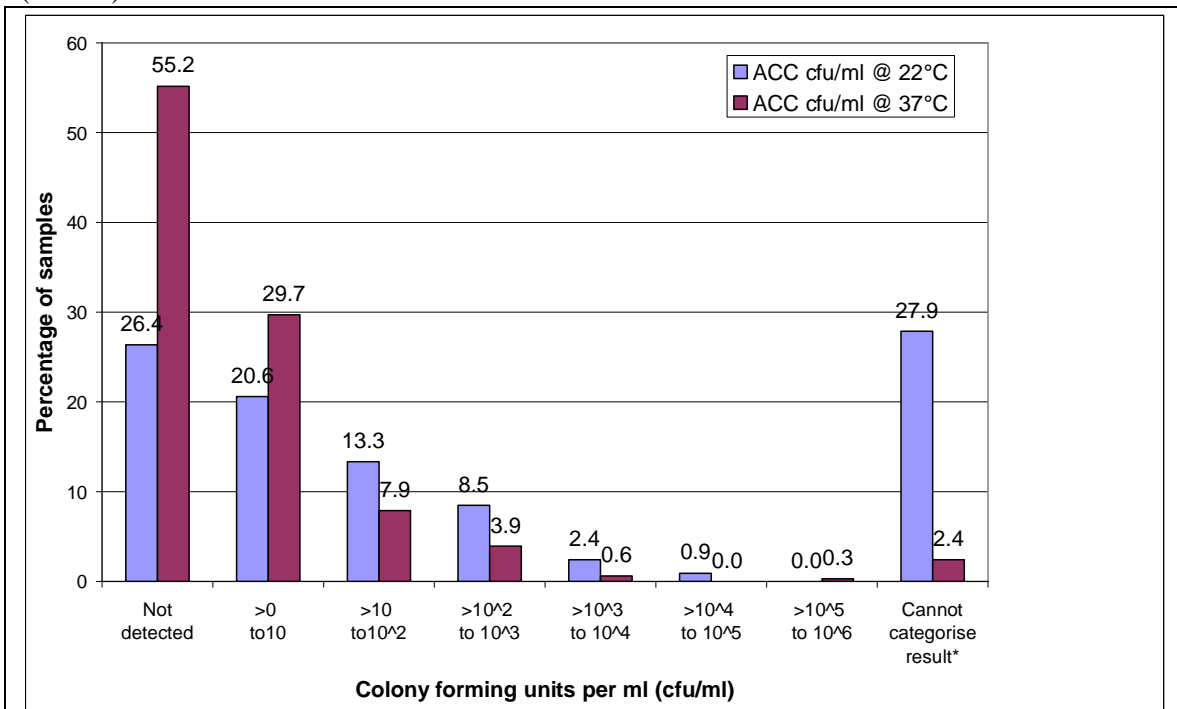
Aerobic Colony Count (ACC) Results

A. Natural Mineral Water (NMW) and Spring Water (SW)

The results of ACC at 22°C and 37°C for the NMW samples are presented in Figure E1. These results provide a snapshot of the microbial load and are dependent on the initial bacterial count in the source water for each bottle and the time lapsed since the water was bottled.

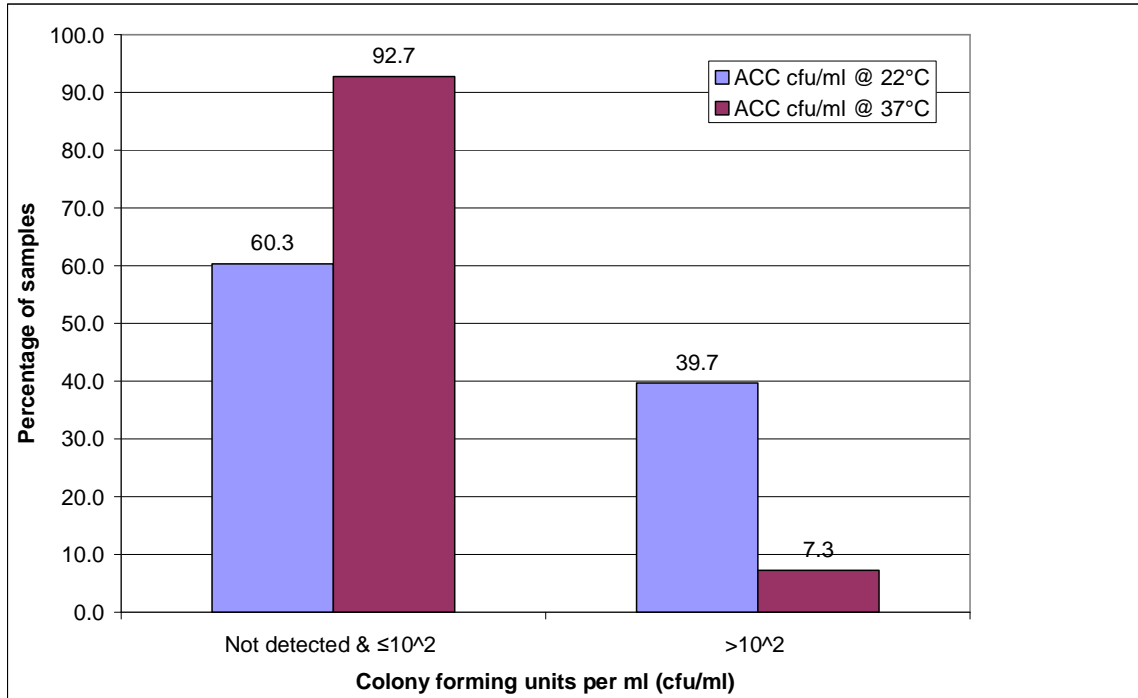
Approximately half of the samples analysed at 37°C (55.2%) and a quarter of the samples analysed at 22°C (26.4%) had no colonies detected. 27.9% of the results for samples analysed at 22°C and 2.4% for those analysed at 37°C were reported as ‘greater than’ as opposed to ‘equal to’ a specific number and were therefore labelled as ‘Cannot categorise result’ in Figure E1. As all of these samples were >100 cfu/ml we can report that overall 39.7% of all NMW samples analysed at 22°C and 7.3% of all those analysed at 37°C were >100 cfu/ml (Figure E2).

Figure E1: Results of aerobic colony count (ACC) at 22°C and 37°C of NMW samples (n=330)



*Cannot categorise result: These were samples which were reported as ‘greater than’ as opposed to ‘equal to’ a specific number. See text for further details.

Figure E2: Results for NMW samples (n=330) analysed for aerobic colony count (ACC) at 22°C and 37°C which were $\leq 10^2$ cfu/ml or $> 10^2$ cfu/ml



B. Spring Water (SW)

The results of ACC at 22°C and 37°C for the SW samples are presented in Figure E3. These results provide a snapshot of the microbial load and are dependent on the initial bacterial count in the source water for each bottle and the time lapsed since the water was bottled.

40.2% of samples analysed at 37°C and 20.1% of those analysed at 22°C had no colonies detected. 34.4% of the results for samples analysed at 22°C and 4.2% for those analysed at 37°C were reported as ‘greater than’ as opposed to ‘equal to’ a specific number and were therefore labelled as ‘Cannot categorise result’ in Figure E3. As all of these samples were >100 cfu/ml we can report that overall 59.3% of all SW samples analysed at 22°C and 15.9% of all those analysed at 37°C were >100 cfu/ml (Figure E4).

Figure E3: Results of aerobic colony count (ACC) at 22°C and 37°C of SW samples (n=189)

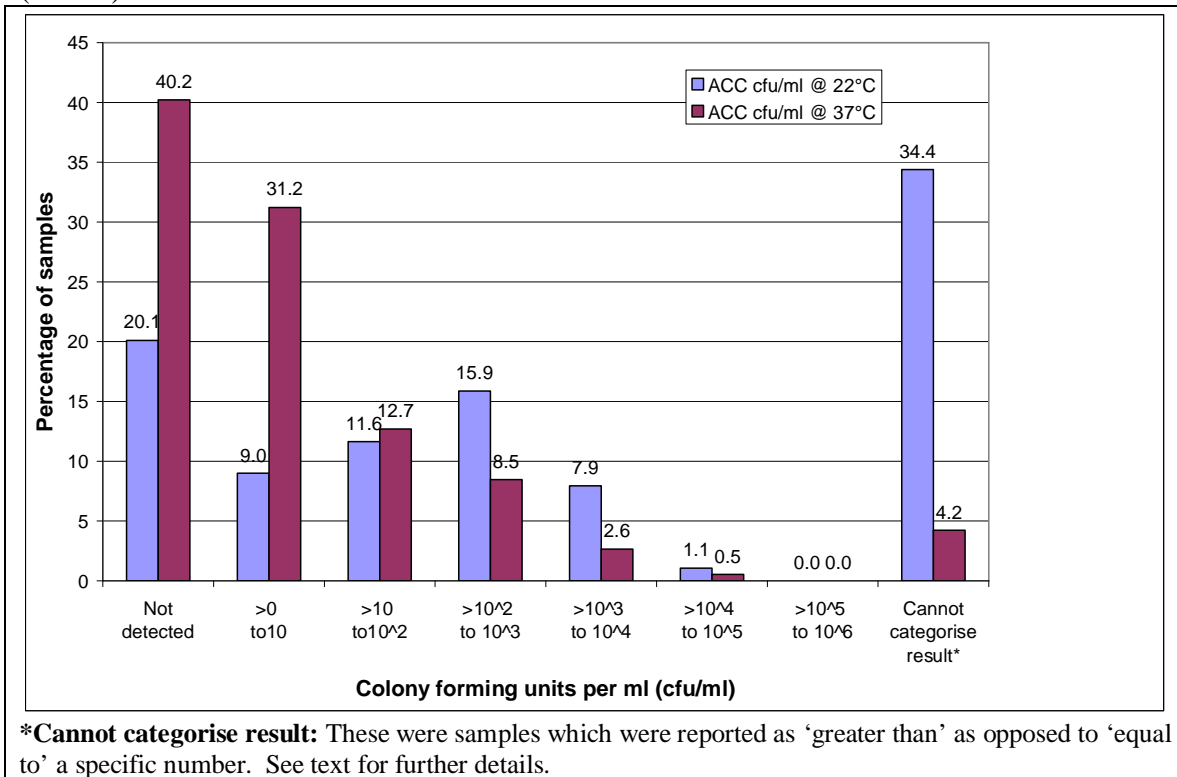
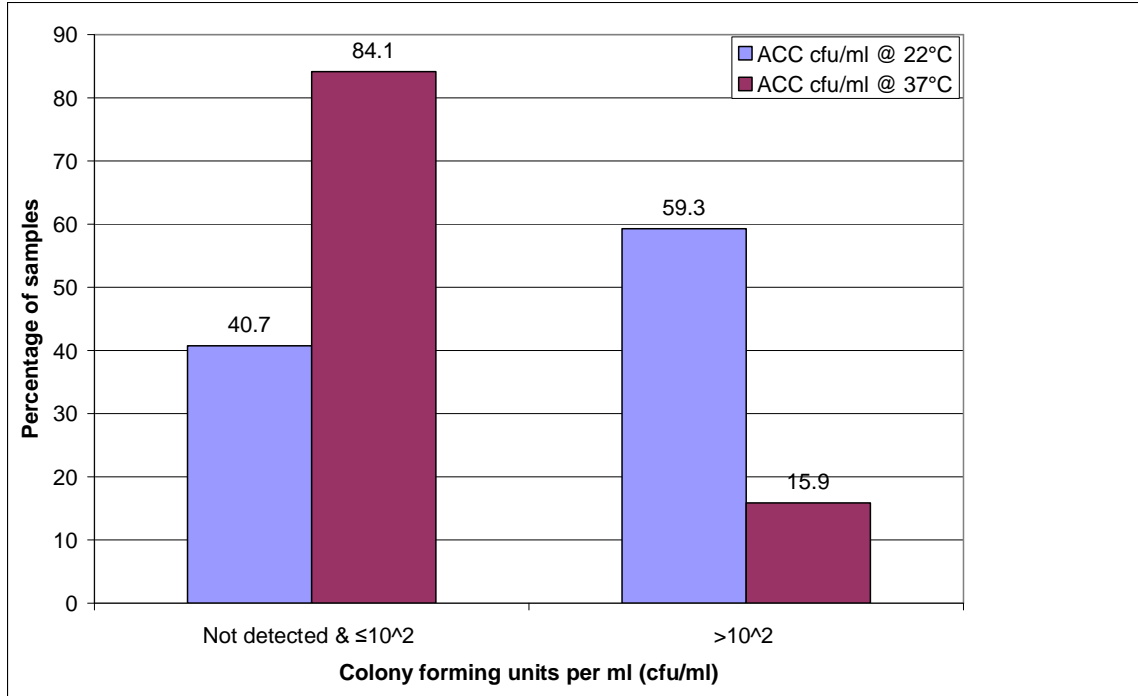


Figure E4: Results for SW samples (n=189) analysed for aerobic colony count (ACC) at 22°C and 37°C which were ≤ 100 cfu/ml or >100 cfu/ml

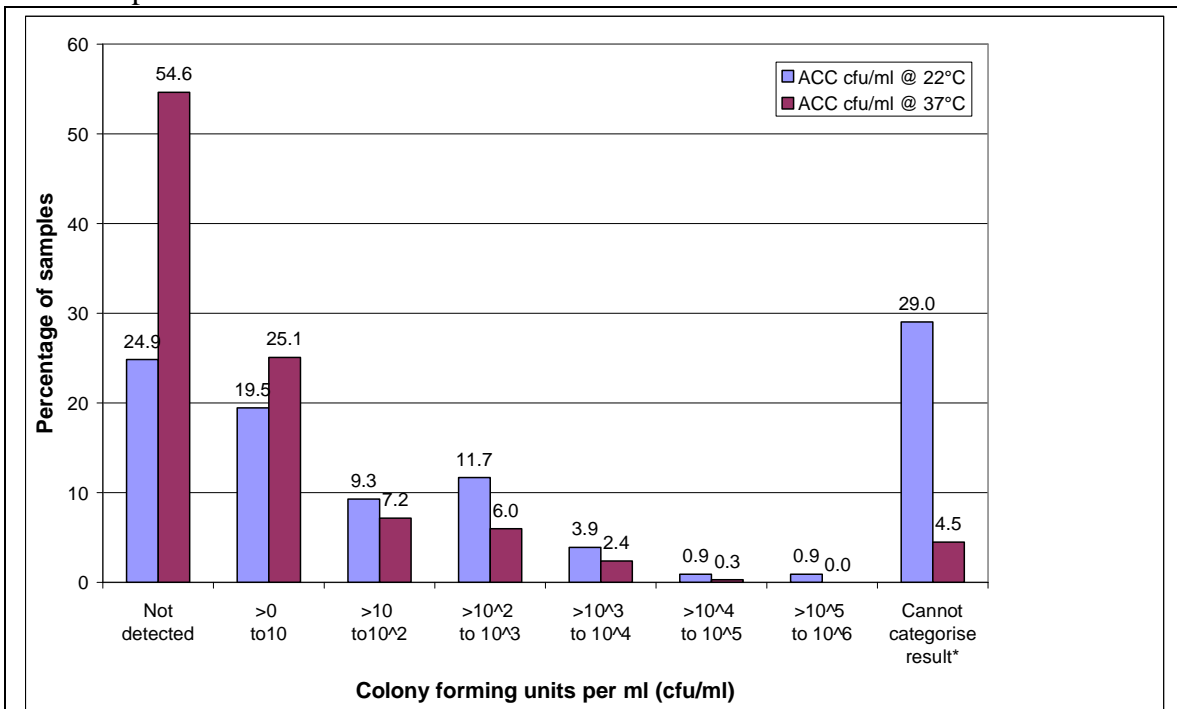


C. Other Water (OW)

The results of ACC at 22°C and 37°C for the OW samples are presented in Figure E5. These results provide a snapshot of the microbial load and are dependent on the initial bacterial count in the source water for each bottle and the time lapsed since the water was bottled.

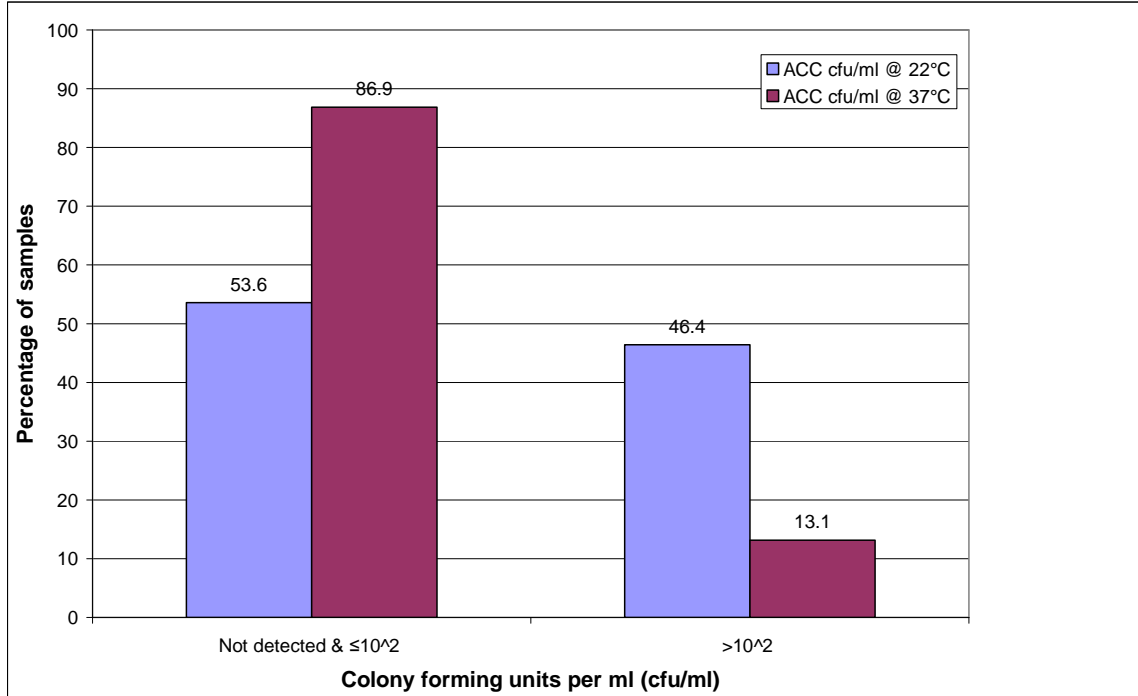
54.6% of samples analysed at 37°C and 24.9% of those analysed at 22°C had no colonies detected. 29.0% of the results for samples analysed at 22°C and 4.5% for those analysed at 37°C were reported as ‘greater than’ as opposed to ‘equal to’ a specific number and were therefore labelled as ‘Cannot categorise result’ in Figure E5. As all of these samples were >100 cfu/ml we can report that overall 46.4% of all OW samples analysed at 22°C and 13.1% of all those analysed at 37°C were >100 cfu/ml (Figure E6).

Figure E5: Results of aerobic colony count (ACC) at 22°C (n=334) and 37°C (n=335) of OW samples.



*Cannot categorise result: These were samples which were reported as ‘greater than’ as opposed to ‘equal to’ a specific number. See text for further details.

Figure E6: Results for OW samples analysed for aerobic colony count (ACC) at 22°C (n=334) and 37°C (n=335) which were ≤ 100 cfu/ml or >100 cfu/ml



D. Uncategorised water

The results of ACC at 22°C and 37°C for the uncategorised samples are presented in Figure E7. These results provide a snapshot of the microbial load and are dependent on the initial bacterial count in the source water for each bottle and the time lapsed since the water was bottled.

57.4% of samples analysed at 37°C and 26.2% of those analysed at 22°C had no colonies detected. 35.5% of the results for samples analysed at 22°C and 2.8% for those analysed at 37°C were reported as ‘greater than’ as opposed to ‘equal to’ a specific number and were therefore labelled as ‘Cannot categorise result’ in Figure E7. As all of these samples were >100 cfu/ml we can report that overall 59.8% of all uncategorised samples analysed at 22°C and 22.2 % of all those analysed at 37°C were >100 cfu/ml (Figure E8).

Figure E7: Results of aerobic colony count (ACC) at 22°C (n=107) and 37°C (n=108) of uncategorised samples.

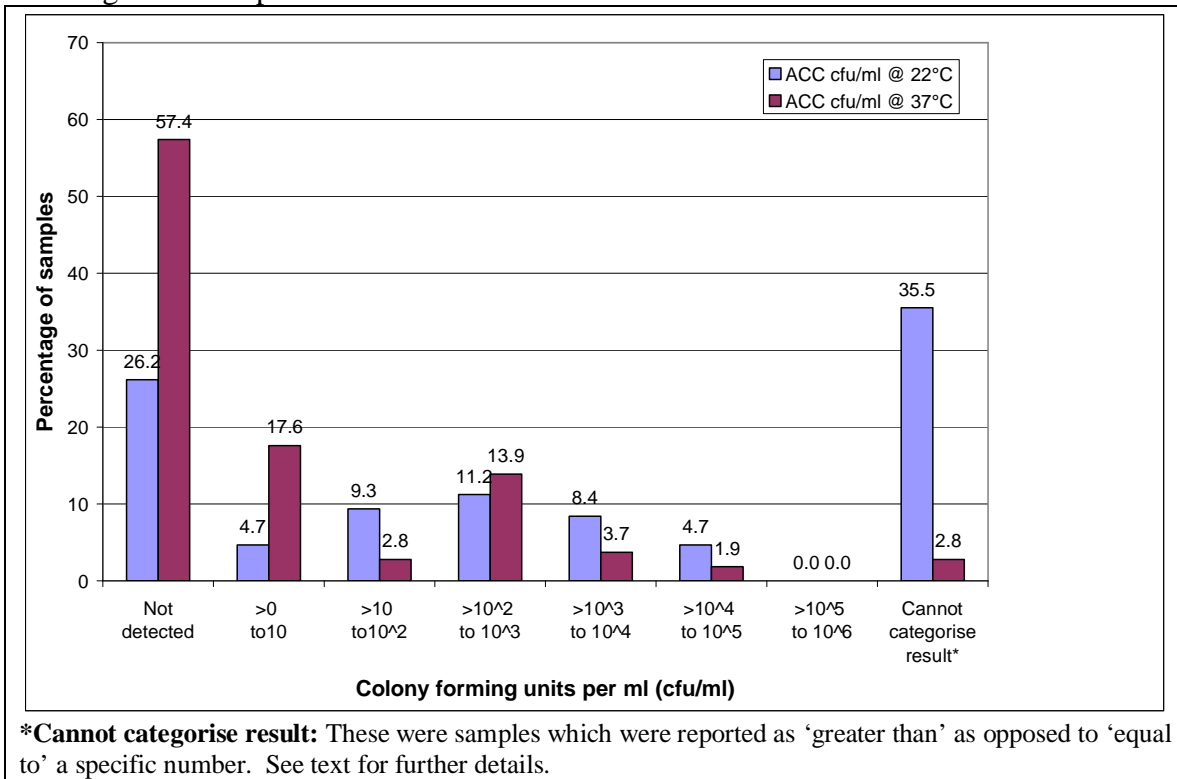


Figure E8: Results for uncategorised samples analysed for aerobic colony count (ACC) at 22°C (n=107) and 37°C (n=108) which were ≤ 100 cfu/ml or >100 cfu/ml.

