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The history and use of HPC in drinking-water quality management

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3.1 INTRODUCTION

As civilizations developed, it became evident that water, especially good quality water, was necessary for their advancement. For centuries, good water was defined as water that was clear, pleasant to the taste and not malodorous. Good food had similar requirements. However, both contaminated water and food were still the causes of countless deaths. Outbreaks of cholera and typhoid occurred for centuries, but the role of water in these outbreaks was not demonstrated until 1849–1854. John Snow identified water as the source of a cholera outbreak in London and became the father of modern epidemiology. Even at the time of Snow, smell, appearance, taste and chemical analysis were the only analytical tools that the water analysts had to determine the wholesomeness of drinking-water. Too often they were wrong, and outbreaks were frequent.

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By the end of the 19th century, with the development of bacteriology, culture media and the gelatin plate, it became possible to obtain what appeared to be quite accurate counts of germs by counting the number of colonies developing on these plates within a defined set of conditions. The simplicity of the method was such that it was rapidly put to use by the 19th-century sanitarians. Air, water, soil, food, humans and animals were all studied to determine where and how germs lived, as they were apparently responsible for a wide variety of waterborne and foodborne diseases.

We are now in the early 21st century, and, reading the accounts of these 19th-century sanitarians, there is a striking resemblance between our so-called modern problems and the problems they had to resolve. The questions they raised are the same ones that we are discussing now. In terms of water quality, it is quite fascinating to observe that the orders of magnitude of the numerical values used to define good quality water have remained the same. While much has been written on the subject of water bacteriology, the books of Hamlyn (1990) and Prescott and Winslow (1904) provide a magnificent view of early water bacteriology.

3.2 GERMS AND DISEASE: FROM DISCOVERY TO CULTIVATION

Counting microbes is an exercise that has been taking place since the advent of the microscope. Through his simple single-lens microscope, Antoni van Leeuwenhoek in 1673 was probably the first to see microbes. Others followed, but the poor resolution of their lenses did not offer a very precise view of the bacterial world. By the 1830s, quality achromatic objectives had been developed and microscopes were being made that opened a new world to the eyes of the bacteriologists.

In England, John Snow demonstrated that water played a significant role in cholera outbreaks. In 1854, another severe epidemic of cholera occurred in London. Through painstaking documentation of cholera cases and correlation of the comparative incidence of cholera among subscribers to the city's two water companies, Snow showed that cholera occurred much more frequently in customers of the water company that drew its water from the lower Thames, which was contaminated with London sewage, than in customers of the other company, which obtained its water from the upper Thames.

By 1861, Louis Pasteur had disproved the spontaneous generation theory, and he later demonstrated the link between germs and disease. Robert Koch described a mechanism whereby a disease such as cholera was spread: it was

excreted in faeces, was transported to water and then infected those who subsequently drank the water. A similar mode of transmission was later described for typhoid fever, and subsequent interest in the role of water in the transmission of disease was thus initially focused on these two infections.

In 1872, Ferdinand Cohn developed a bacterial culture medium containing ammonium salts and yeast ash complemented with various sugars; this medium provided the bacteriologist with a tool to test the growth requirements of bacteria. In 1881, Koch published a paper in which he described the gelatin plate method — a revolution in bacteriology — for growing pure cultures of bacteria. Obtaining pure cultures was now easier, and the enumeration of germs was possible. In 1882, the use of agar instead of gelatin was introduced, and in 1887, Richard Julius Petri invented the petri dish.

The birth of microbiology in 19th-century Europe was the basis for water and food microbiology and the first step in understanding the role of water and food as vehicles for the transmission of disease (Beck 2000). Growing germs was not an easy task, but bacteriologists were discovering the basic nutrients that these germs needed to grow. They now had the tools to study water and food. These methods were rapidly adopted by sanitarians from all countries on both sides of the Atlantic.

3.3 KOCH: ASSESSING FILTER EFFICIENCY AND SETTING LIMITS

Water filtration had been introduced in 1804 in Scotland as a means of producing better quality water for a clothes-washing industry. Water from the River Cart was passed through trenches filled with stones before being passed through a ring-shaped settling chamber. The water was clear and contained less suspended solids, thereby not soiling the clothes. Because the process produced more water than needed, the surplus was sold to the town inhabitants. The product was of good quality, and others rapidly followed this lead. The first sand filters were developed by James Simpson in England in the 1820s.

By the end of the century, it was common to have filtered water, and the protective effect of this filtration was dramatically demonstrated in 1892 in Germany. The Elbe River, near Hamburg, was contaminated by sewage from a cholera-stricken refugee camp. Hamburg experienced an outbreak that killed over 7000 people, while the city of Altona, using the same water but filtered, experienced only a few cases unrelated to the water. Koch investigated this outbreak and exchanged information with water analysts all over Europe. He suggested that filtering was better than not filtering, that careful management of filters was better than poor management, that even careful management could

not protect the public absolutely, and finally that "when all was said and done, he, personally, would rather not drink this filtered water at all. Yet one had to live with uncertainty, to trust something less than rigorous demonstration, and be satisfied with estimates of risk."

Overall, the rudimentary bacteriological analysis of all types of water during these catastrophic outbreaks led the early sanitarians to better quality source water and water treatment and thereby a reduction in waterborne outbreaks. It also pointed out the value of water treatment to protect public health. Koch proposed a limit of 100 cfu/ml as the objective to protect public health. This value was proposed to assess the "purity" of source water and, hence, its usability as a source of drinking-water. It was also proposed as a means to assess water filtration efficiency in order to produce safe drinking-water from "impure" sources. It was only later that the same value was also used to evaluate the efficiency of the disinfection of drinking-water by chlorine and other means. For several years, Koch had also been analysing waters and counting colonies that grew in agar at "blood heat," thinking that these organisms would likely include pathogens.

The value proposed by Koch has remained unchanged until today and has apparently remained aimed at the protection of public health by a more or less direct evaluation of source water and treatment. Since the discovery of water bacteria and their relation to disease, the United Kingdom and the USA approached the plate count with two different philosophies, as described in sections 3.4 and 3.5 below.

3.4 WATER MICROBIOLOGY: THE UNITED KINGDOM EXAMPLE

3.4.1 Early water microbiology

Although Robert Koch had demonstrated the use of solid media for culturing bacteria in London in 1881, it was only in 1884 that British water analysts and sanitarians began to take interest in it and *The Lancet* published a lengthy description of the plate culturing method, noting that "the numbers and nature of the organisms present in a sample of water may be estimated and ascertained" using this technique. The book of Hamlyn (1990) presents an account of British efforts to understand water quality and control waterborne diseases; it has been an inspiration for this section.

The simplicity of the technique was its greatest problem, as it tempted those with little or no bacteriological training to try the process. Many recognized that the method required skills in order to obtain accurate results, but the British

sanitarians realized that bacteriological examination ought to be carried out on a widespread basis for the examination of water supplies and for ascertaining the relative value of domestic filters.

One of the early advocates of bacteriology was Percy Frankland (1858–1946), who worked with his father at the School of Mines, where he was an assistant in the water laboratory. After learning of the plate culturing method at an exhibition in London, he visited Koch's laboratory to master this new method. Frankland used the method from 1885 onward to measure the numbers of bacteria in water and evaluate the efficiency of filtration. He observed what is now well established: filters are effective for the removal of bacteria, they lose their efficiency with time, and smaller filters become clogged and support bacterial growth. In his words, this was an "exceedingly beautiful and ingenious test for ascertaining the number of individual organisms present in a given water," with "little value" for distinguishing bacterial types (quoted in Hamlyn 1990). Overall, more and more people agreed that plate cultures showed the value of filtration in removing microorganisms.

Many questions on these methods were also raised at the time; surprisingly, they are still familiar even to modern water bacteriologists.

Was gelatin-peptone the best medium? What was the sensitivity of the medium for waterborne pathogens? Comparison of media and their ability to support pathogens became a familiar exercise. Lower counts on nutrient-rich media and upon incubation at "blood temperature" were observed, as was the poor growth of pathogens on nutrient-poor media. By the late 1890s, most analysts would insist that use of several media was necessary if one was to speak confidently on the bacterial content of a water.

What was the relationship between the bacteria in the water and the bacterial counts and species growing on the plates? Having observed the bacteria under the microscope and recorded different counts of bacteria on different culture media, scientists realized that the number of colonies that grew on the plate could not be regarded as the true total number of bacteria in the water.

What do the bacterial counts indicate? To the British, it became rapidly evident that these determinations indicated what would be the probable fate of pathogens gaining access to the water supply and their potential to reach the consumers. A method of treatment reducing the largest proportion of organisms of all kinds would also be the most likely to reduce pathogens should they be present.

Interpretation of the data was becoming controversial: some questioned the bacterial counts, since microbial populations would rapidly increase in suitable conditions. Koch had suggested a standard of 100 colonies/ml as the limit of acceptability, but what would be the risk of drinking that "acceptable" water if

after a week in a container it contained 10 000 colonies/ml? The same question is raised today.

The use of plate counts became widespread, and an incubation temperature of 18–22 °C became the norm, with daily examination of plates for up to five days (Horrocks 1901). Additional counts of bacteria after incubating a second set of plates for 40–48 h at 36–38 °C were recommended in 1904 (Royal Institute of Public Health 1904), as these bacteria were considered more likely to represent those that could grow in the human body and, therefore, could be indicative of faecal contamination, although it was recognized that many other naturally occurring bacteria were also capable of growing at this temperature (Savage 1906). During this time, counts at 18–22 °C or 20–22 °C were typically conducted using nutrient gelatin plates, and those at 37 °C were conducted using nutrient agar plates (Royal Institute of Public Health 1904; Savage 1906).

3.4.2 Early use of heterotrophic plate counts (HPC)

Formal guidance on the bacteriological examination of water supplies and the interpretation of results was first published by the United Kingdom Ministry of Health in 1934 (Anonymous 1934) as what was to become universally known as "Report 71." The recommended method involved dispensing 1-ml aliquots of water, mixing with nutrient agar and incubation of one set of plates at 20–22 °C for three days and another set at 37 °C for two days, which, apart from a change of medium, has continued to today and is widely used throughout the world. The number of bacteria enumerated at 20–22 °C was said to give "some indication of (1) the amount of food substance available for bacterial nutrition and (2) the amount of soil, dust and other extraneous material that had gained access to the water," whereas the count at 37 °C "affords more information as to dangerous pollution," as "the organisms developing at this temperature are chiefly those of soil, sewage, or intestinal origin, and their number, therefore, may be used as an index of the degree of purity of the water" (Anonymous 1934). The report also stated that the colony count of a single sample had comparatively little significance and that "it is difficult to state limits which, if exceeded, involve unfavourable comment on the hygienic quality of the water." The ratio of the count at 22 °C to that at 37 °C was said to be helpful in explaining sudden fluctuations, with high ratios being associated with bacteria of clean soil or water saprophyte origin and, therefore, of "small significance" (Anonymous 1934). This approach was reaffirmed in the second edition of Report 71, published five years later (Anonymous 1939).

3.4.3 Guidance on the use of HPC

Experience gained over the next 17 years, however, led to a change of emphasis in the third edition of Report 71 (Anonymous 1956; Society for Water Treatment and Examination 1956), which stated that "although plate counts at 22 °C and 37 °C reflect by an increase in the numbers, particularly at the higher temperature, the access of faecal pollution, they are not now usually employed for this purpose." Their principal use was now one of a more general detection of "any form of contamination," maintaining their role as indicators and not a health parameter in their own right. The report presented a review of the agar plate count (written by E. Windle Taylor, then Director of Water Examination of the Metropolitan Water Board, London), which discussed the wide variability of numbers of bacteria from differing water types and sources and technical aspects of the method, concluding that "high plate counts at either temperature, even if confirmed, do not necessarily indicate that a water is a danger to health." They were, however, "undesirable since the presence of large numbers of bacteria in water may cause food spoilage." The key value of plate counts was their use in assessing the efficacy of water treatment processes, providing an "estimate of the general hygienic quality of a water" (particularly with regard to food production), and "a rising plate count may give the earliest sign of pollution" (e.g., in wells) (Anonymous 1956). This interpretation of the value of plate counts was reiterated in the fourth and fifth editions of Report 71 (Anonymous 1969, 1982), which also stated that "colony counts are not essential for assessing the safety of domestic water supplies." The fourth edition also introduced yeast extract agar as the medium of choice for the enumeration of colony counts and confirmed an incubation time of only 24 h for counts at 37 °C, introduced in the 1956 third edition. The 1982 fifth edition also noted that "organisms which grow best at 37 °C usually grow less readily in water and are more likely to have gained access from external sources" and that "a sudden increase ... would call for immediate investigation since it might be an early sign of more specific or serious pollution" (Anonymous 1982). All reference to the use of HPC to potentially indicate faecal contamination had been dropped.

3.4.4 Interpretation of HPC levels

Significant strides in the understanding of microbial behaviour, particularly with regard to heterotrophic bacterial populations, in water supplies during the 1980s and 1990s were reflected in the sixth edition of Report 71, published in 1994 (Standing Committee of Analysts 1994). The three key areas where plate counts were of value, outlined in the 1956 third edition, remained, but multiplication of bacteria within distribution systems due to available nutrients (assimilable

organic carbon) in the water or fixtures and fittings and the growth of biofilms and their potential role in taste and odour problems were also recognized (interestingly, a relationship between available nutrients and bacterial growth had been alluded to in the 1934 and 1939 editions of Report 71, but not since). The report stated that "in practice, changes in the pattern of colony counts of samples from a given water supply are usually more significant than the actual numerical count of any particular sample" and that "the counts themselves have little direct health significance." The report recognized that some potentially opportunistic pathogens (e.g., *Pseudomonas aeruginosa* and *Aeromonas* sp.) may be part of the colony count population, and "their appearance in large numbers in water indicates that conditions in the distribution system have become suitable for growth as opposed to survival of these organisms." However, it concluded that without evidence of faecal contamination, "elevated colony counts should not be viewed with concern in terms of the health of the population as a whole." Regular enumeration of colony counts from a distribution system did, however, provide useful data with which to assess any long-term trends in the general microbial quality of drinking-water.

This interpretation of the use of colony counts is retained in the seventh edition of the guidance (Standing Committee of Analysts 2002a, 2002b), prepared with regard to the new United Kingdom legislation (Anonymous 2000) arising from the 1998 European Union (EU) Directive (European Union 1998). The guidance re-emphasizes that "it is not the absolute numbers of colony count bacteria enumerated from a supply that are of importance, but whether there are significant changes or long-term trends in those numbers." Although the requirement to enumerate colony counts at 37 °C is no longer stipulated in the EU Directive, it has been retained in the United Kingdom legislation and is still considered to be of some value, "in that it can provide an early indication of a significant deterioration in quality before coliform bacteria or other indicator bacteria are detected (for example, due to ingress into a distribution system)" (Standing Committee of Analysts 2002a).

This edition also reintroduced the option of incubating 37 °C plates for up to 48 h (Standing Committee of Analysts 2002b), as had been the norm prior to 1956, and is also in agreement with the International Organization for Standardization (ISO) standard ISO 6222:1999 (ISO 1999), stipulated by the 1998 EU Directive (European Union 1998) as the method to be used. The lower incubation range in the ISO standard is 22 °C ± 2 °C, which is a wider range than the 20–22 °C historically used in the United Kingdom and recommended by the United Kingdom guidance (Standing Committee of Analysts 2002b).

When the United Kingdom adopted the first EU Directive on drinking-water (European Union 1980), the guideline values for plate counts (10/ml at 37 °C

and 100/ml at 22 °C) were not formally included. Instead, the regulations stated that there should be "no significant increase over that normally observed" (Anonymous 1989a). Guidance from the regulators (Anonymous 1989b) stated that "continuous review is needed of colony counts" and that further investigation should be taken if "there is a sudden and unexpected increase in a colony count, particularly the 37 °C count, compared with that normally found" or "there is a significant trend of increasing colony counts in the supply over a period of a few years." Both the current EU Directive (European Union 1998) and United Kingdom regulations (Anonymous 2000) do not set numerical standards or guideline values for colony counts, which are defined as indicator parameters, but state that there should be "no abnormal change." This is in keeping with the approach that colony counts are an operational tool for the management of water quality in distribution systems. It does, however, beg the question as to what an "abnormal change" is. There is currently no official guidance on this in the United Kingdom (or Europe), and, consequently, there are several approaches that have been adopted by water suppliers.

Many suppliers employ simple numerical values for an indication of an abnormal change in counts from regulatory samples; some have based these values on the guideline values of the first EU Directive (European Union 1980), whereas others have adopted higher values (e.g., >10, >20, >50, >100, >200, >300, >500, >1000 cfu/ml at 22 °C or 37 °C). These values generally serve as triggers to review previous data and make an assessment of any significance of the increase. Some have established arbitrary levels of increase ranging from 0.5 log to >2.3 log increases over previous results. This has the advantage that it automatically takes into account the natural rise and fall in heterotrophic bacterial populations that occur during the seasons. A few suppliers have adopted a statistical approach (several others indicated that they were also investigating a statistical approach), based upon a comparison with mean counts. The time base of the data for which mean counts are calculated can vary, depending upon the seasonal variation in the counts and the frequency of analysis, with some covering the previous few weeks and others a period of a year or more (e.g., 20 times a three-year mean, >3 standard deviations from previous six results, >1.5 times a 12-month rolling mean or the >98th percentile of rolling annual mean).

3.4.5 Current use of HPC in the United Kingdom

The principal use of the data gathered from regulatory monitoring is to monitor trends or deterioration (in terms of rising counts) in quality, and some suppliers have targeted trend monitoring with data from service reservoirs. Other uses of the data are chlorine management, modelling of microbial populations,

performance assessment of treatment works, assessment for planned maintenance of infrastructure (e.g., cleaning of service reservoirs) and secondary indicators of quality following isolation of coliforms or other primary indicators. Most suppliers have regular review periods, typically monthly, half-yearly or annually, some undertaking reviews on both a regular basis and by an unusual result. Most of these reviews are undertaken on an informal basis, but several have a formal programme, some linked in with their quality assurance procedures (e.g., ISO 9002 — ISO 1994).

Undertaking plate counts as part of a suite of analyses when responding to claims of ill health is the most widespread use, with most suppliers doing counts at both 37 °C and 22 °C, but a few only at 37 °C. The rationale is that plate counts may indicate a significant event within the distribution system, not that HPC bacteria may be related to ill health. Plate counts are also widely used when investigating complaints of off-tastes or odours, as changes in HPC populations may indicate proliferation of biofilms, which can be associated with microbially mediated generation of some organoleptic compounds (Standing Committee of Analysts 1998). Operational plate counts are also commonly used as part of acceptance criteria for new mains prior to being put into supply and in assessing water quality following mains rehabilitation work.

The use of counts of heterotrophic bacteria has, therefore, a long history in the United Kingdom. The count at 22 °C has been used as a general indicator of water quality since 1885. The count at 37 °C was originally introduced with the belief that it could indicate potential faecal contamination, but this was soon disregarded, although it is still used for operational management in the United Kingdom, despite being dropped in the EU Directive.

Coliform bacteria are also no longer regarded as indicators of faecal contamination, but are of use as indicators of general microbial quality. This acknowledges that some coliform bacteria may be part of the natural bacterial flora in water and proliferate in biofilms. Coliforms are also considered useful for monitoring treatment processes and assessing the disinfection of new or repaired mains (Standing Committee of Analysts 2002a).

3.5 THE AMERICAN PERSPECTIVE ON THE PLATE COUNT

3.5.1 Early water bacteriology in the USA

It did not take long for these “new methods” to cross the Atlantic, and by 1904, the first edition of *Elements of Bacteriology with Special Reference to Sanitary Water Analysis* (Prescott and Winslow 1904) contained most of what is today

considered modern bacteriology. The principles of this book, re-edited until 1946 (sixth edition), are still pertinent to the discussions that we have today. A similar book, written by William G. Savage, entitled *The Bacteriological Examination of Water-Supplies* and published in 1906 in London, presents the British story and the state of knowledge in England at that time.

In the preface to the first edition of their book, Prescott and Winslow (1904) summarize the context:

> Bacteriology has long since ceased to be a subject of interest and importance to the medical profession merely, but has become intimately connected with the work of the chemist, the biologist, and the engineer. To the sanitary engineer and the public hygienist a knowledge of bacteriology is indispensable.
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> In the swift development of this science during the last ten years perhaps no branch of bacteriology has made more notable progress than that which relates to the sanitary examination of water. After a brief period of extravagant anticipation, and an equally unreasonable era of neglect and suspicion, the methods of the practical water bacteriologist have gradually made their way, until it is recognized that, on account of their delicacy, their directness, and their certainty, these methods now furnish the final criterion of the sanitary condition of a potable water.
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> The treatment of the subject in the many treatises on General Bacteriology and Medical Bacteriology is neither special enough nor full enough for modern needs. The classic work of Grace and Percy Frankland is now ten years old; and even Horrocks' valuable "Bacteriological Examination of Water" requires to be supplemented by an account of the developments in quantitative analysis which have taken place on this side of the Atlantic.

The plate count had been applied to a variety of waters, and what were considered "normal values" were being confirmed. Prescott and Winslow (1904: pp. 8, 9, 10) wrote:

> With regard to what may be considered normal values for rain we have no very satisfactory figures. Those obtained by Miquel (Miquel, 1886) during the period 1883-1886, showing that rain contains on the average 4.3 bacteria per c.c. in the country (Montsouris) and 19 per c.c. in Paris, are probably lower than would be yielded by the present methods of examination ... In the larger streams several conditions combine to make the bacterial number lower ... A good river-water under favorable conditions should thus contain only a few hundred bacteria ... The student will find numerous analyses of natural waters in Frankland's classic work (Frankland, 1894). He notes, for example, that the Lake of Lucerne contained 8 to 51 bacteria per c.c., Loch Katrine 74, and the Loch of Lintralthen an average of 170. The water of

> Lake Champlain examined by one of us (S.C.P.) in 1896 contained on an average 82 bacteria per c.c. at a point more than two miles out from the city of Burlington ...

Many observers had believed that groundwaters were nearly free from bacteria, because often no colonies appeared on plates counted after the usual incubation period of two days. Longer periods of incubation yielded higher counts, occasionally in very large numbers, and the multiplication of bacteria in the samples after collection or bottling had been observed. The conclusion was that all water types contained bacteria and that one needed to find the correct medium to grow these organisms. However, for the sanitary bacteriologist, the limits were different (Prescott and Winslow 1904: pp. 19–20):

> That the customary methods for determining the number of bacteria do not reveal the total bacterial content, but only a very small fraction of it, becomes apparent when we consider the large number of organisms, nitrifying bacteria, cellulose-fermenting bacteria, strict anaerobes, etc., which refuse to grow, or grow only very slowly in ordinary culture media, and which, therefore, escape our notice.
>
> ... the numbers obtained by the ordinary procedure were only from 5 to 50 per cent of those obtained by the use of Heyden's Niahrstoff agar. For practical sanitary purposes, however, our methods are fairly satisfactory. Within limits, it is of no great importance that one method allows the growth of more bacteria than another.
>
> When we are using the quantitative analysis as a measure of sewage pollution only two things are essential. First, media should be of standard composition, so that results obtained at different times and by different observers may be comparable ... Secondly, it is desirable that the section of the total bacterial flora which we obtain should be thoroughly representative of that portion of it in which we are most interested — the group of the quickly growing, rich-food-loving sewage forms. In this respect our meat gelatin-peptone appears to be unrivalled ... To emphasize this difference with constancy is all that we require of a method for practical work.

The conditions of sample conservation had also been investigated and had shown that there "is first a slight reduction in the number present, lasting perhaps for six hours, followed by the great increase noted by earlier observers. It is probable that there is a constant increase of the typical water bacilli, overbalanced at first by a reduction in other forms, for which this is an unsuitable environment." These results made it obvious that samples must be examined shortly after collection and that they must be kept cool during their storage. At this time, the recommendation was that "It is, therefore, necessary to adhere strictly to the recommendations of the A.P.H.A. Committee that the interval between sampling and examination should not exceed twelve hours in

the case of relatively pure waters, six hours in the case of relatively impure waters, and one hour in the case of sewage."

The incubation period was, as it is still today, the subject of much discussion. American and German bacteriologists counted the number of colonies after 48 h, while the French were using longer incubation periods and obtaining higher counts. The Americans considered that the longer incubation period was in fact obscuring the difference between good and bad waters, because the fast-growing bacteria were associated with sewage originating from the human intestine. Whatever the conditions of the test, Prescott and Winslow (1904: p. 35) considered the interpretation of this simple test as a complex process:

> The information furnished by quantitative bacteriology as to the antecedents of a water is in the nature of circumstantial evidence and requires judicial interpretation. No absolute standards of purity can be established which shall rigidly separate the good from the bad. In this respect the terms "test" and "analysis" so universally used are in a sense inappropriate. Some scientific problems are so simple that they can be definitely settled by a test. The tensile strength of a given steel bar, for example, is a property which can be absolutely determined. In sanitary water analysis, however, the factors involved are so complex and the evidence necessarily so indirect that the process of reasoning much more resembles a doctor's diagnosis than an engineering test.

On either side of the Atlantic, classes of water were being defined. In France, as early as 1891, Miquel classified waters as follows: "water with less than 10 bacteria per c.c. was 'excessively pure,' with 10 to 100 bacteria, 'very pure,' with 100 to 1000 bacteria, 'pure,' with 1000 to 10 000 bacteria, 'mediocre,' with 10 000 to 100 000 bacteria, 'impure,' and with over 100 000 bacteria, 'very impure.'" In Germany, water containing fewer than 100 bacteria was presumably from a deep source and uncontaminated by surface drainage; one with 500 bacteria was open to suspicion; and one with over 1000 bacteria was presumably contaminated by sewage or surface drainage (Sternberg 1892).

By 1904, it was also clear that organisms growing at body temperature and those fermenting lactose were not numerous in normal waters, with total counts rarely exceeding 50/ml. However, when polluted waters were examined, counts of acid producers on "litmus-lactose-agar" plates were likely to run into hundreds. The method, therefore, was considered "one of the most useful at the disposal of the bacteriologist. It yields results within twenty-four hours, and the conclusions to be drawn from it are definite and clear" (Prescott and Winslow 1904).

The Americans did not consider the plate count as part of their water regulations until recently.

3.5.2 Measuring HPC microorganisms in the USA

In the USA, bacteriological methods for the analysis of water were proposed by the American Public Health Association in collaboration with the American Water Works Association in the first edition of what was to become known as "Standard Methods" (APHA 1905). From its first edition in 1905 until its 20th edition in 1998 (APHA *et al.* 1998), the methods have been modified on several occasions. The basic plate count on nutrient gelatin at 20 °C for 48 h was used for several years and was later modified to include agar as the solidifying agent and a shorter incubation period of 24 h, which remained the main method until the 1980s. Because food microbiologists using the plate count had standardized the method at 35 °C for water, food and dairy products, this became the recommended temperature of incubation in the 10th edition (APHA *et al.* 1955). By 1985, several variations were in use (i.e., pour plate, spread plate and membrane filtration), and the plate count was referred to as the "heterotrophic plate count" or HPC.

By the end of the 1980s, American bacteriologists had developed culture media that could detect a higher proportion of heterotrophic bacteria (Reasoner and Geldreich 1985). The media were developed to maximize bacterial recoveries; they yielded higher counts when incubated for 5–7 days at 20 °C or 28 °C and permitted the examination of larger sample volumes by membrane filtration methods. Because of the limited inclusion of fewer nutrients at higher concentration, these media detect higher numbers of fewer different species of the diverse heterotrophic bacterial population.

By the mid-1980s, the Americans, who had no standard for the plate count at the time, had several groups review the "plate count" and its implications. The bacterial plate count for analysing water had been used in combination with the coliform test for a number of years and appeared in 1914 as a US drinking-water standard with a limit of 100 cfu/ml. As experience accumulated with the total coliform test and plate count test, the fact emerged that the latter provided unreliable information on the presence of bacterial pathogens in drinking-water. For this reason, the test was not included in the succeeding US Public Health Standards of 1925 and thereafter. While there was no requirement for plate counts as a drinking-water standard even in the 1970s, the US Environmental Protection Agency (EPA) stated its belief that "the standard plate count is a valid indicator of bacteriological quantity of drinking water, and recommends that it be used in appropriate cases in conjunction with the coliform tests as an operational tool" (US EPA 1975). At the same time, the National Academy of Sciences (1977) stated that "the Standard Plate Count is a valuable procedure for evaluating the bacterial quality of drinking water."

Numerical values were more difficult to define. In 1989, the US EPA addressed the issue in one of its rules and set the level to 500 cfu/ml at 35 °C, as a non-health-related secondary standard, mainly for considerations relating to interference with the coliform test. Both the "Surface Water Treatment Rule" (US EPA 1989a) and the "Coliform Rule" (US EPA 1989b) contained requirements for monitoring the HPC, as a high HPC is associated with false-negative coliform tests when lactose-based media are employed and as HPC is a surrogate indicator for chlorine residuals in distribution systems. The method chosen for measuring HPC was left to the water utility, but the numerical objective was the same.

According to Reasoner (1990), HPC is a useful tool for 1) monitoring the efficiency of the water treatment process, including disinfection; 2) obtaining supplemental information on HPC levels that may interfere with coliform detection in water samples collected for regulatory compliance monitoring; 3) assessing changes in finished water quality during distribution and storage and in distribution system cleanliness; 4) assessing microbial growth on materials used in the construction of potable water treatment and distribution systems; 5) measuring bacterial regrowth or aftergrowth potential in treated drinking-water; and 6) monitoring bacterial population changes following treatment modifications, such as a change in the type of disinfectant used.

3.5.3 Interference with the total coliform assay

Documents prepared by the US EPA by the mid-1980s show that the Americans were mainly focusing on the interference of high plate counts with the coliform assay and the presence of opportunistic pathogens in the bacterial population defined by the plate count (US EPA 1984).

Reasoner and Geldreich (1985), who were the developers of the new culture media for HPC, presented the various uses of the HPC: evaluation of the treatment process(es), primarily disinfection; evaluation of the levels of HPC that may interfere with coliform compliance; evaluation of the quality of finished treated drinking-water and of distribution system cleanliness; and evaluation of the potential for biofilm formation.

The Americans relied mainly on total coliform and thermotolerant (faecal) coliform assays to assess their water quality, and the preferred methodology was membrane filtration. Setting total coliforms as the key method to all water analysis, they integrated the HPC, not for its operational value, but mainly to limit the interference with total coliform enumeration. Investigations had suggested that high HPC densities (i.e., over 500/ml as enumerated on standard plate count [SPC] media) could substantially interfere with membrane filtration tests that were lactose-based, but that this phenomenon may not occur

consistently. Overcrowding on membrane filter plates appeared to be a major reason for atypical coliform colonies. In addition to interference with coliform analysis methodology, large numbers of SPC bacteria were also suggested to reduce coliform levels during sample transit and storage. Geldreich *et al*. (1978) collected 613 samples from flushes of dead-end water mains in Cincinnati, Ohio. Data analysis demonstrated a correlation between excess SPC densities and desensitization of the membrane filtration method. They concluded that the method was less efficient when SPC densities exceed 500–1000 cfu/ml.

As these studies indicate, American water bacteriologists were essentially working with data suggesting the presence of interfering factors in some waters; whether these were intrinsic factors of a physicochemical nature (organic and inorganic precipitates) or related to a predominance of certain bacteria types has not been fully explained.

However, general guidelines were formulated. Values of less than 100 cfu/ml were considered achievable for all systems. Values from 100 to 500 cfu/ml, anticipated during seasonal increases or at certain locations in the system (dead end, low residual), would suggest a need for flushing. Values greater than 500 cfu/ml would suggest poor microbial quality. The last category was not defined in terms of action to be taken. In other cases, 5- to 10-fold increases over normal levels were set as a guideline to prompt an investigation (US EPA 1984).

3.6 OPPORTUNISTIC PATHOGENS AND HEALTH EFFECTS

On this theme appear the most controversial discussions of the last part of the 20th century. Using various media designed specifically for this task, it is possible to grow various pathogens, such as *Legionella*, *Mycobacterium*, *Escherichia coli*, *Campylobacter* and many other species, from water samples. While none would dispute the fact that most, if not all, bacterial pathogens are "heterotrophic bacteria," many equated the plate count with these pathogens. The following citation is a typical mixed-message example of what can be found in texts of the period: "Many members of the SPC population have longer survival times than fecal contaminants in water, and many (e.g., *Mycobacterium*, *Bacillus*, and *Clostridium*) are more resistant to disinfectant than fecal pathogens."

Some bacteria counted in the HPC are certainly more resistant to disinfection; *Bacillus* spores have been described as a good indicator of treatment efficiency. Mycobacteria are very slow growers, are very difficult to grow and would not be counted on an HPC plate. Clostridia are strict anaerobes

and therefore would not be found in the population growing on the plate count media and would not be "members of the SPC."

The list of colony-forming bacteria on HPC media and identified in water is long and illustrates the diversity of the environment: *Acinetobacter*, *Actinomycetes*, *Alcaligenes*, *Arthrobacter*, *Corynebacterium*, *Micrococcus*, *Moraxella*, *Pseudomonas*, *Aeromonas*, *Citrobacter*, *Enterobacter*, *Yersinia*, *Hafnia*, *Klebsiella*, *Serratia*, etc. (Payment 1999). The same bacteria are found often in large numbers in food products.

While there have been several studies of the bacterial species found in water, the identification of bacterial isolates from the environment has always been impaired by a poor database. It is highly probable that many of the isolate identifications reported in the literature over the years are incorrect. Comparisons of various available identification systems have shown that the same isolate will be identified differently according to the database used. In the 1980s, many identifications were made employing clinical systems for which the database was not appropriate for environmental strains. Molecular methods have changed our views of the "species," and we should at least question many of the bacterial identifications in the literature. Some may be correct to the genus level and a few to the species level, but none can define the pathogenicity of these bacteria, as we will see further.

From the 1980s until now, many researchers in the water industry have equated the genus or species names of the bacterial isolates found in the plate count to those of isolates implicated in clinical disease. Few water bacteriologists were involved in clinical microbiology, and the isolates named were equated to pathogens and disease. Few pondered the true complexity of pathogenicity: among the myriad of *E. coli* strains that can be found in water, only a few are pathogenic. In a clinical setting, it is only through the identification process down the serological pattern that clinicians can identify the true pathogen and the relationship to disease in a particular environment. Finding *E. coli* in urine has a different significance than finding it in stools. Isolating a strain of *Campylobacter* or *Salmonella* in stools does not necessarily mean that it is the cause of disease (de Wit *et al*. 2000).

For some true pathogenic strains (i.e., strains that had been isolated from diseased individuals and shown to cause disease according to Koch's principles), oral infective dose data were available. As many of the isolates from water samples had the same identification (genus, species), most water microbiologists took the quantum leap: their isolates could also be pathogens, and even bacteria implicated very rarely in clinical disease became foes.

An EPA-supported study compared influent and effluent SPC densities for 25 point-of-use devices and generally found about a log or more increase in the effluent. It was concluded that there was a risk to immunocompromised

individuals: "Among the opportunistic pathogens which grow on these filters are *Pseudomonas aeruginosa* and *Flavobacterium* species. The proliferation of these pathogens may pose a health risk to compromised individuals consuming the effluent water" (Calderon and Mood 1988, 1991).

Many scientists went further, and statements such as the following were common: "A positive relationship between SPC densities and waterborne disease outbreaks has been reported in a few cases, but published data are sparse" (US EPA 1984: p. 33) or

> There are cases on record where a change in the SPC density has signaled the imminence of a waterborne outbreak. In 1926, for example, Hanover, Germany, experienced flooding of wells by highly contaminated river water. A substantial increase in SPC numbers was not initially accompanied by positive coliform counts. Hanover experienced 40,000 cases of gastroenteritis followed by an outbreak of typhoid fever (Muller, 1977). Muller (1977) also reported that similar observations occurred at Pforzheim in 1919 and at Gelsenkirchen in 1889. [US EPA 1984: pp. 48–49]

Those were sufficient reasons to jump to the conclusion that the correlation was universal. It failed to acknowledge that HPC numbers were often high in the absence of any overt disease and that one could not demonstrate a correlation. The epidemiological value of the anecdotal evidence is poor, but the statement influenced a large number of water specialists. In fact, it fell in the same category as coliforms and *E. coli*: both are used as indicators of treatment of faecal contamination, but, to many plant operators, they are disease-causing organisms.

The conclusion to most of the debates and of committees formed to study the risk is summarized by this statement: "While there is no conclusive evidence to date that opportunistic pathogens have caused disease via the waterborne route, there is strong supportive evidence this is true. Since virtually everyone in the U.S. is exposed to SPC bacteria whenever they consume or otherwise use potable water, including the compromised population, this is an area of concern" (US EPA 1984: pp. 59–60).

This statement, in its simplicity, fails to acknowledge the major source of exposure to HPC bacteria: food. As is shown in section 3.8 below, the HPC rapidly found its way in the food industry, where it has become a tool to study food degradation. The food industry faced the same problem and came up with a very different solution. Many food products could contain more than 1 000 000 cfu/ml before they began to deteriorate to a point where they were spoiled. This

was defined by the food industry not in terms of public health but in terms of food quality.

Several studies were also concerned with the presence of virulence factors in HPC bacteria (Lye and Dufour 1991; Payment *et al*. 1994; Edberg *et al*. 1997; Drinking Water Inspectorate 1998). They recognized that there were bacteria in drinking-water that contained recognized virulence factors, but that they were in small numbers and that only animal studies or epidemiological evidence could demonstrate the significance of these bacterial strains. Recent studies in immunocompromised animal models determined the true meaning of these virulence factors detected *in vitro*, and these studies have shown that none of the HPC bacteria isolated from drinking-water and expressing various virulence factors were pathogenic for immunocompromised mice (Stelma *et al*. 2002).

3.7 HEALTH EFFECTS: EPIDEMIOLOGICAL STUDY

Epidemiology again became a tool to answer the questions raised and the potential risks. The immunocompromised population had been growing rapidly with the spread of HIV/AIDS, and, with the advances in medicine, there was now an increasing number of transplant patients artificially immunosuppressed by drugs.

Because of lack of faith in tap water quality, a large number of households were using various point-of-use devices based on activated charcoal to remove chemical contaminants from water. It did not take long to show that these filters supported bacterial growth and that the effluent often contained more bacteria than the incoming water (Geldreich *et al*. 1985). Heterotrophic bacteria were using the accumulated organics in the activated charcoal filter matrix to proliferate. These could be the source of opportunistic pathogens, or the filters themselves might support the growth of incoming bacterial pathogens.

The first epidemiological studies on possible health effects were conducted in the USA by Calderon and Mood (1988, 1991) on a large number of households using various point-of-use or point-of-entry devices based on granular activated charcoal. High HPC levels were observed, but there were no apparent health effects demonstrated.

A prospective epidemiological study on the health effects of tap water was conducted in Canada. It included 600 families, 300 of which had been provided with reverse osmosis units to remove contaminants from their tap water (Payment *et al*. 1991a). The installation of the device had a protective effect for gastrointestinal disease transmitted by tap water: the individuals in the filter group experienced 35% fewer gastrointestinal episodes that those in the unprotected group. HPC counts at 20 °C and 35 °C had been obtained from the reverse osmosis units on several occasions, and it was thus possible to correlate

the level of disease in the family with the HPC counts at 37 °C (Payment *et al*. 1991b). The apparent association was driven by a few outliers in the data set and probably gave this result apparent, but unlikely, statistical significance.

In a second study, the same group (Payment *et al*. 1997) used bottled water as a means of testing the health effects of drinking-water. Highly purified bottled water and tap water (from a water filtration plant) were given to two groups of families; a third group consumed tap water, and a fourth group consumed water from a tap equipped with a bleeder valve that continuously purged the system, thereby preventing stagnation and regrowth of heterotrophic organisms. The results confirmed that tap water was a significant source of gastrointestinal disease in the population (17–40%). While the bottled purified (reverse osmosis filtered and ozonated) water remained relatively free of bacteria, the water collected at the water treatment plant supported an active HPC growth within a few days, as would be observed in the distribution system upon stagnation. The HPC population grew from 2 to 30 000 cfu/ml (25 °C) and from 0 to 985 cfu/ml (37 °C) in a week, with extremes at 1 400 000 cfu/ml (25 °C) and 895 000 cfu/ml (37 °C). The individuals who had consumed water with high bacterial counts had reported less illnesses than those consuming tap water. They had the same level of illness as those consuming pure bottled water with very few bacteria. The group of families consuming water from a tap equipped with bleeder valves had a level of gastrointestinal illnesses slightly higher than those in the tap water group. This indicated that regrowth of bacteria in drinking-water was not the source of the observed illnesses.

These studies all suggested that high bacterial counts from bacteria developing in tap water or bottled water were not contributing to an increase of gastrointestinal illnesses in a normal population (i.e., a population composed of individuals of all ages and normally healthy).

3.8 HPC BACTERIA IN FOOD

Historically, the bacterial plate count occupies a strong position as an analytical tool for determining the microbial quality of a variety of raw and processed food products, such as meats, dairy products and canned foods. It was among the first of the definitive scientific methods employed for quality control in such products, and its use continues today as the major tool for their bacteriological examination.

The European Economic Community (EEC) directives for various food products would appear totally unacceptable to most water bacteriologists; however, this is what we eat everyday. A few examples from various EEC directives or the United Kingdom guidelines (PHLS Advisory Committee for

Food and Dairy Products 2000) are presented in Table 3.1 and illustrate the order-of-magnitude difference between the two worlds.

Table 3.1. EEC directives and United Kingdom guidelines for the microbial quality of food products

Product	Microorganisms	Maximum value
1) EEC directives		
Egg products (Directive 89/437/EEC)	Aerobic mesophilic bacteria Enterobacteriaceae	100 000 cfu/g or ml 100 cfu/g or ml
Pasteurized drinking milk (Directive 92/46/EEC)	Plate count at 21 °C	50 000 cfu/g
Minced meat (Directive 94/65/EEC)	Aerobic mesophilic bacteria *E. coli* (non-pathogenic)	5 000 000 cfu/g 500 cfu/g
Frozen milk-based products (Directive 92/46/EEC)	Coliforms Plate count	100 cfu/g 50 000 cfu/g
2) United Kingdom guidelines		
Pork pies, sausage roll, raw pickled fish, mousse	Aerobic colony count 30 °C, 48 h	<10 000 cfu/g
Ice cream, pizza, cakes and pastries (without dairy cream), mayonnaise, cooked vegetables	Aerobic colony count 30 °C, 48 h	<100 000 cfu/g
Sliced beef and poultry, seafood meals, cakes and pastries (with dairy cream), dried fruit, coleslaw	Aerobic colony count 30 °C, 48 h	<1 000 000 cfu/g
Sliced ham, smoked fish, prepared mixed salads, sandwiches and filled rolls	Aerobic colony count 30 °C, 48 h	<10 000 000 cfu/g

A survey conducted in 1999 in Australia provides an interesting perspective on self-serve salad bars (West Australia State Health Laboratory Service 1999):

> The median SPC value was 185,000 cfu/g. Forty-six (63.9%) samples had an SPC less than 1,000,000 cfu/g, nineteen (26.4%) had an SPC between 1,000,000 and 10,000,000 cfu/g, seven samples had an SPC greater than 10,000,000 cfu/g. There were no samples with an SPC greater than 100,000,000 cfu/g.

The same is true in the USA, as the following citation from a Massachusetts requirement for frozen desserts illustrates (Massachusetts Department of Public Health 1999):

The bacteriological limits for frozen desserts set forth in 105 CMR 561.009 are 10 coliform colonies per gram and 50,000 standard plate count (SPC) per gram. It is the responsibility of local boards of health to enforce monthly testing and reporting requirements for frozen dessert establishments, as well as to take appropriate actions when bacteriological violations have been found ...

Q. Does a standard plate count (SPC) slightly above the standard of 50,000 colonies per gram present a public health concern?

A. The limit of 50,000 SPC is intended as a guideline. Usually the SPC represents harmless organisms, especially if there are no coliforms associated with the sample. Spoilage organisms usually begin to affect the frozen dessert product in numbers much greater than 50,000. It usually takes counts of 1,000,000 or greater to create spoilage. According to 105 CMR 561.009, exceeding 50,000 once is not considered a violation. When a SPC is only slightly high, i.e., 150,000, consider the company's track record.

3.9 MANDATORY OR GUIDELINE HPC VALUES IN THE 1970S AND 1980S

After the initial impetus, bacteriological tests became a simple routine measurement for the control of water treatment; in many countries, the plate count was not defined by mandatory values. Most regulatory texts simply stated that the absence of pathogens was expected and that control was to be achieved using coliform bacteria.

Apart from semantics and terminology, what was meant by the "total count of bacteria" in water did not change much, the basic definition being "the number of bacterial colonies produced on an agar plate under defined medium and incubation conditions." Heterotrophic bacteria include all those bacteria that can use organic nutrients for growth. The aquatic environment contains an extremely diverse flora of these organisms. All known primary and secondary bacterial pathogens, whether transient or indigenous, that are spread by the water route are heterotrophic. No single analytical tool can satisfactorily detect and enumerate all heterotrophic bacteria or measure their full range of metabolic activities.

In addition to the term "standard plate count," many designations have been used: "heterotrophic plate count," "total viable count," "total count," "plate count," "total bacterial count," "bacterial count," "water plate count" and "colony count," as well as "aerobic, mesophilic viable bacteria." Some are used in the water industry, some in the food industry, others in biology. The "standard plate count" term was used in the USA until the 15th edition of

Standard Methods (APHA *et al.* 1980) and was changed in the 16th edition (APHA *et al.* 1985). The nomenclature currently refers to the "heterotrophic plate count" as defined in the 20th edition of Standard Methods (APHA *et al.* 1998).

Several countries adopted mandatory values for the colony counts of water (Table 3.2). They are still used in most of these countries (see chapter 12), and they are very similar to the values suggested by Koch at the end of the 19th century. The European Union (1980) did recommend guideline values for total bacterial counts in drinking-water of 10 cfu/ml at 37 °C and 100 cfu/ml at 22 °C. Even if this appears convenient in its simplicity, there are differences in the defined conditions of medium and incubation, as well as other analytical parameters, from one country to another, as illustrated in Table 3.3. These guideline values, however, were dropped in the 1998 directive (European Union 1998).

Even if setting guideline values appears convenient, the impact of these differences on results is not really known. When setting an international level for any type of water (especially in point-of-use devices or bottled waters), these differences could significantly affect any decision made on the basis of the numerical results obtained. Furthermore, the rationale for using a particular value is rarely apparent in the texts supporting the regulations. WHO *Guidelines for Drinking-water Quality* still provide such information (WHO 1996).

3.10 STANDARDS AND GUIDELINES IN THE 1990S

The current standards or guidelines for HPC bacteria in tap water vary slightly between different nations. In general, HPC monitoring is used as a tool to gain information on the water treatment process and the general bacteriological quality of the water leaving the water treatment plant and within the distribution system. Examples of specific guidelines for drinking-water (tap or bottled) from several countries and agencies have been reviewed in chapter 12.

3.11 CONCLUSIONS

The HPC was the basic test that led public health officials and water treatment engineers to improve the quality of drinking-water. The plate count was rapidly replaced in most regulations by coliform testing, which provided a better indication of the sanitary quality of the water. In the early 1900s, the HPC was being used only as a secondary test to further assess treatment efficiency. While several technological developments led to media capable of detecting higher numbers of bacteria, very little was done to assess the variations in the bacterial subpopulations isolated on these different media at different temperatures.

Table 3.2. Some mandatory colony count values in Europe in 1977 (adapted from Muller 1977)

	Application	Mandatory value (counts/ml)	Temperature (°C)
Poland	Public supply	100	20
		25	37
	Well water	100	37
		500	20
Yugoslavia	Treated water	10	37
	Underground (raw)	100	37
	Surface (raw)	300	37
Romania	Public water supply (>70 000 consumers)	20	??
	Other water supplies	100–300	??
Switzerland	Raw water	100	??
	Raw water (distributed)	300	??
	Immediately after treatment	20	??
	Distribution system	300	??
Netherlands	Tap water	100	20
Sweden	Tap water	100	20
Germany (GDR)	Tap water	100	20
Spain	Good quality water	50–65	37
	Tolerable water	100	37
France		No guide	
United Kingdom		No guide	
USA		No guide	

The guideline values proposed by Koch at the end of the 19th century are very similar to those set by today's regulations in many countries. Various rationales have been proposed to justify the choice of specific guideline values: a few considered possible health effects, some considered attainable values, others found that HPC interfered with other tests, some found it useful for various tasks, many simply followed suggested guidelines.

The concerns relating to the presence of opportunistic pathogens within the bacterial population detected in the plate count have essentially been put to rest by several studies. Recent literature suggests that direct health effects are improbable, especially when compared with the extremely high plate counts that have been considered acceptable in food products. The historical background in the food industry provides ample evidence that these bacteria are mostly

Table 3.3. Example of the diversity of methods for the determination of plate count in drinking-water as set by water regulations in various countries during the 1980s (modified from NATO 1984)

		Canada	Netherlands	Norway	FRG	Sweden	France	UK
Procedure		Pour plate	Pour plate	Pour plate	Pour plate	Pour plate	Pour plate	Pour plate
Samples	ml/plate	As required	1	1	1	1	1	1
	Replicate	2	2	2	2	2	2/dilution	1–2/dilution
	Dilution	As required	As required	As required	As required	As required	As required	As required
	Diluent	Phosphate-buffered distilled water	0.1% peptone water	0.9% NaCl	Sterile tap water	Phosphate-buffered distilled water	Distilled water or Ringer's solution ¼×	Ringer's solution ¼×
Media	Medium	Tryptone glucose yeast extract agar	Tryptone glucose yeast extract agar	Tryptone glucose yeast extract agar	Meat extract peptone agar	Meat extract peptone agar	Yeast extract agar	Yeast extract agar
	Sterilize	15 min, 121 °C	15 min, 121 °C	15 min, 121 °C	20 min, 120 °C	20 min, 120 °C	20 min, 118 °C	20 min, 115 °C
	Incubation	48 h 35°C	48 h, 37 °C 72 h, 22 °C	72 h, 20 °C	44 h, 20 °C	48 h, 22 °C	24 h, 37 °C 72 h, 20–22 °C	24 h, 37 °C 72 h, 20–22 °C
Counting	Aids used	Quebec colony	Automatic colony	Hand lens	Hand lens (8×)	Hand lens	Hand lens	Hand lens

harmless, non-pathogenic organisms. That they can cause disease in extreme conditions remains possible (e.g., cuts, surgery, immunosuppression, etc.): many microorganisms given an opportunity to enter the human body can cause great harm. This is not the case when they are ingested.

In 2002, after more than 125 years, the case for setting HPC levels in drinking-water still remains an open question in the minds of many. This brief review of the HPC in history suggests that the main cause for concern has been the focus of water bacteriologists on the sanitary consequences of the HPC. Early bacteriologists had rapidly determined that in the absence of faecal contamination, the role of the HPC was not as an indicator of public health risk. Food bacteriologists, faced with the same problem, also accepted that HPC bacteria were mainly nuisance organisms, and they set guidelines that are orders of magnitude higher than those for drinking-water. Therefore, the future use of HPC in water testing appears to be mainly as a validation and verification test, with no direct relationship to public health.

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