残留塩素には遊離残留塩素と結合残留塩素とがあり、両者の合計が全残留塩素である。遊離残留塩素とは、塩素剤が水に溶けて生成する次亜塩素酸（HClO）・次亜塩素酸イオン（OCl^-）のことで反応しやすい状態にあり、それだけ殺菌力も強い。結合残留塩素とはモノクロラミン（NH_2Cl）・ジクロラミン（$NHCl_2$）などアンモニアと結合した酸化力をもつ有効塩素をいい、ゆっくり反応するため殺菌力は弱いが残留性は高い。水道水中の残留塩素については、1957年（昭和32年）成立の水道法第22条を受けて、水道法施行規則第16条で「給水栓（蛇口）における水が、遊離残留塩素を0.1ppm（＝mg/ℓ）（結合残留塩素の場合は0.4ppm）以上保持するように塩素消毒をすること」と定められており、通達で「水の消毒は塩素によるものとする」となっている。また、1993年（平成5年）12月1日から新たな水質基準[2]が施行され、同時に、水質基準とはならなかったものの厚生省の通知によってより質の高い水道水を供給するための快適水質項目と呼ばれる13項目に目標値が設定され、残留塩素は1mg/ℓ程度以下と設定されている。残留塩素量は時間の経過とともに変化するので、その場で分析するのであるが、残留塩素の測定目的は、細菌の増殖抑止の指標である。すなわち、水中の大腸菌や一般細菌の量を短時間で測定できないので、殺菌剤の塩素が残っていればこれらの菌類が増殖していないとの判断から残留塩素を測定し水の飲用安全性の指標としている。なお、残留塩素の基準値については、欧米での設定値は日本が0.1または0.4mg/ℓ以上と下限値を定めているのに対し上限値制をとっており、米・仏では遊離残留塩素の上限値を0.1mg/ℓとしており、日本の下限値制に関しての議論がされている[3]。

通常の浄水方法のうち、沈殿、濾過では水中の細菌類を完全に除去することは難しく、水道水の消毒は、飲料水の安全を確保するため水中の病原生物を完全に除去するとともに配水系統の病原菌汚染から水道水を守るため不可欠なことである。水道水の消毒剤としては、液化塩素、次亜塩素酸ナトリウムまたは次亜塩素酸カルシウム等の塩素剤以外による消毒は認められていない。通常は液化塩素が用いられ減圧気化して注入されているが、小規模浄水場では液化塩素より高価であるが取り扱いや安全性を考慮して次亜塩素酸ナトリウムが使われている。なお、理論的に0℃の液体塩素1ℓは464ℓの塩素ガスを発生する。

塩素を水に注入すると

$Cl_2 + H_2O \rightarrow HCl + HClO$　　(1)

$HClO \rightarrow HCl + O$　　(2)

となり(2)式で単体の激しく反応する酸素（過激酸素）を発生してほかのものに強い酸化作用を及ぼす。この酸素が酸化・漂白したり細菌中の酵素の働きを阻害することにより殺菌を行う。しかし(1)式の次亜塩素酸は強い酸化作用により管を直接に腐食させ、塩酸は水の中のアルカリ分を中和して水を酸性に傾けさせる。

また工場排水や生活排水などによって水が汚れている河川から水道原水を取水して水道水を作るときは、

(1)アンモニア性窒素や有機物等を酸化分解する。

(2)水中にいる一般細菌や大腸菌群などを死滅させる。

(3)藻類や小型動物を死滅させる。

(4)水の中に溶けている鉄やマンガンを不溶性の酸化物として除去する。

のような目的で塩素処理が行われている。

この酸化剤としての塩素は、環境汚染による水質悪化が高まる1960年代から、原水中のアンモニア性チッ素の硝酸化や細菌類を処理するためのいわゆる前塩素処理（現在は中塩素処理に移っている）として多用されてきており河川の汚れの進行とともに使用量が増大している。すなわち、塩素剤はコストがかからず、殺菌力も強力で消毒効果が確実であり、水中に残留することにより消毒効果が長続きするので用いられており、塩素注入はコレラ、赤痢、腸チフスやパラチフス等細菌による消化器系の伝染病の抑止に効果を上げた。つまり、水道水の消毒に使う程度の塩素の濃度では、飲用による人体への影響がなく、塩素は水道水の消毒剤として優れた多くの能力をもっており、各

種の水質汚染物質に対する処理剤としての効果も絶大であるということで使用されている。

一方、残留塩素により水道水は腐食性が高くなり絶えず水と接触している鋼管や銅管などの給水管やタンクの腐食を促進する作用があり、鉄が酸化沈殿し水酸化第二鉄（赤錆）となることが原因で引き起こされる赤水現象が発生することになり、メタリン酸ソーダなどのリン酸塩系やケイ酸塩系の赤水防止剤が使われている。

また、水が土壌透過することにより原水中に含まれる天然物質であるフミン質などの有機物と塩素が反応して発ガン性物質であるトリハロメタンが発生することが問題になっている。また、下水や屎尿の処理水や生活雑排水にも有機物は含まれているから河川水の繰り返し使用する場合には更にトリハロメタンが発生する。トリハロメタンは、メタン（CH_4）の4個の水素のうち3個がハロゲンに置き換わった化合物で、水道水質基準[2]では健康に関連する29項目の中に、総トリハロメタン（0.1mg/ℓ以下）のほかにクロロホルム（0.06mg/ℓ以下）、ジブロモクロロメタン（0.1mg/ℓ以下）、ブロモジクロロメタン（0.03mg/ℓ以下）およびブロモホルム（0.09mg/ℓ以下）のトリハロメタンの4つの成分ごとにも基準値が定められており合計5項目になっている。なお、塩素処理によって生じる有機塩素化合物のうちトリハロメタンは2割に過ぎず、トリハロメタン以外の塩素化合物としてクロロ酢酸類・クロラール類・クロロアセトン類・クロロアセトニトリルが報告されているがまだ5割以上の不明な有機塩素化合物があると報告[4]されている。なお、前述した物質のうちクロロアセトンを除いたものは水質基準の監視項目になり指針値が設定されている。

水道水中の残留塩素濃度は、配水・給水施設の給水領域の末端の給水栓での濃度が下限値以上であることを要しており、配水・給水中にロスが生じるため配水・給水施設における塩素濃度はかなり高いものとなる。従って、配水・給水施設付近の給水栓における塩素濃度は高く、配水・給水施設から離れれば離れるほど低くなる。配水管から分かれて、家庭やビルに引き込まれている管が給水管で、水道とは一戸建住宅の場合、給水栓までつまり水が出るところまでをさす。ビルや共同住宅では水道の給水圧が低いため4階建て以上の建築物においては1階か地下に設けた受水槽に水道の水を貯水し、そこから揚水ポンプにより屋上などの高置水槽に送りそこから各給水栓へ給水するのであるが、水道の責任は受水槽への入り口までであり、その先は使用者や設置者の責任となっており受水槽の容量により維持管理方法が異なる。厚生省による「建築物における衛生的環境の確保に関する法律」施行規則により法の規制を受けるのは、受水槽の容量が10㎥を超えるビルや共同住宅などの水道事業のみから水の供給を受ける給水設備で、簡易専用水道に該当し設置者が法律上の管理義務を負い、施行規則により遊離残留塩素や水質検査が義務づけられている。これらの施設は平成7年末で163,357ヵ所[5]ある。また、受水槽の有効容量10㎥以下の給水施設は法的義務の対象外となっているため管理が不十分なため衛生上の問題が懸念されるものもある。既に述べてきたように、水道水の安全性を確保するために塩素が加えられており、塩素が残留していることが水をそのまま飲用できる証しといえる。河川水を原料とする水道水は、季節や天候などによってまた原料水源の混合割合によって原水の水質が変化し、これに対応する浄化を行うため当然のことながら水質が変化し、残留塩素は年間を通じて一定ではなく変動をしている。ビルや共同住宅では、ほとんどの施設において水槽からの給水方式をとっており、給水栓の残留塩素は一戸建住宅のように配水・給水施設からの距離や供給される水道水の水質によるというよりも、受水槽から給水栓までの状態に依存している。実際の給水栓での遊離残留塩素は少なめで0.2～0.4mg/ℓ、多めで0.5～0.8mg/ℓのところが多いといわれているが、給水栓での水質が受水槽入口の水質と同様であるか、給水栓で規定値が確保されているか等は安全性の面から非常に重要なことである。本学においても、ミネラルウォーター等のボトル水を飲用している者もいるが、多くの者が水道水を飲用に供しており、その安全性の確保のため管理・検査がなされている

と思うが知らされてはいない。そこで、本学における給水栓での残留塩素濃度や季節変動を測定し、水道水の貯留による残留塩素への影響を調べた。

1. 測定について

残留塩素濃度の測定は、東京女子医科大学看護短期大学の3階の研究室の給水栓からの水道水を対象とし、測定器はUSEPA（米国環境保護庁）承認のDPD法を用いた残留塩素計を用いた。掘割式の地階を含め6階建ての校舎の給水施設は、地下受水槽の容量が22㎥、屋上の上屋に設置されている鉄板性高置水槽の容量は5㎥である。測定は1996年（平成8年）7月から翌年7月までで、通水開始からの通水量に対する残留塩素濃度の測定以外は、開栓時に高置水槽から給水栓までに滞留していた水をすべて排出させるに十分な通水を行い、高置水槽内の水道水が直ちに給水栓に至るようにしてから採水した。通水量に対する残留塩素濃度の測定においては、採水間隔と採水量は通水開始から累積通水量2ℓまでは0.2ℓずつ、2ℓ以降は0.4ℓずつ採水した。また図には採水開始時の通水量と採水終了時の通水量の中間の値を代表値として表した。

2. 残留塩素の変動

図1は、通水開始からの通水量に対する残留塩素濃度を示したもので、(A)は1月、(B)は4月の測定例である。○印は遊離残留塩素、●印は全残留塩素、□印は水温を表す。なお、前述した通り全残留塩素と遊離残留塩素の差が結合残留塩素となる。残留塩素は、いずれも通水1ℓあたりまでは低濃度で、以降急激に変化している。給水幹管内の残留塩素濃度が高くても、給水栓付近の枝管内の水道水の残留塩素量は低く、特に遊離残留塩素は0.1mg/ℓという下限値よりはるかに低い0.02mg/ℓ以下であった。著者は水道水中の鉄濃度から通水開始直後の1ℓの水は使用しないほうがよいことを示した[9]が、残留塩素からも同じことがいえる。また、3ℓ位通水すると残留塩素濃度は長時間通水したときの水すなわち高置水槽内の水に近いレベルになっていた。

図2は5月下旬から6月下旬までの1カ月間の残留塩素濃度と水温の変化を示したものである。水温は約5度変化しているが、遊離残留塩素は0～0.02mg/ℓとほぼ一定の値をとっていた。全残留塩素は0.06～0.14mg/ℓの間で変化しているが、変化に傾向はみられず0.1mg/ℓ前後の濃度となっており、全体的には大きな変化はみられないといえる。

表1は、残留塩素濃度および水温の季節による変動を示したものであり、表の値は各月の下旬に測定したものである。冬季の1月のみ遊離残留塩素0.1mg/ℓ以上、全残留塩素0.5mg/ℓ以上という施行規則を満たしているが、他は基準値よりはるかに低い値であり、春季の4月では既に下限値の三分の一程度の濃度であり、これより以降さら

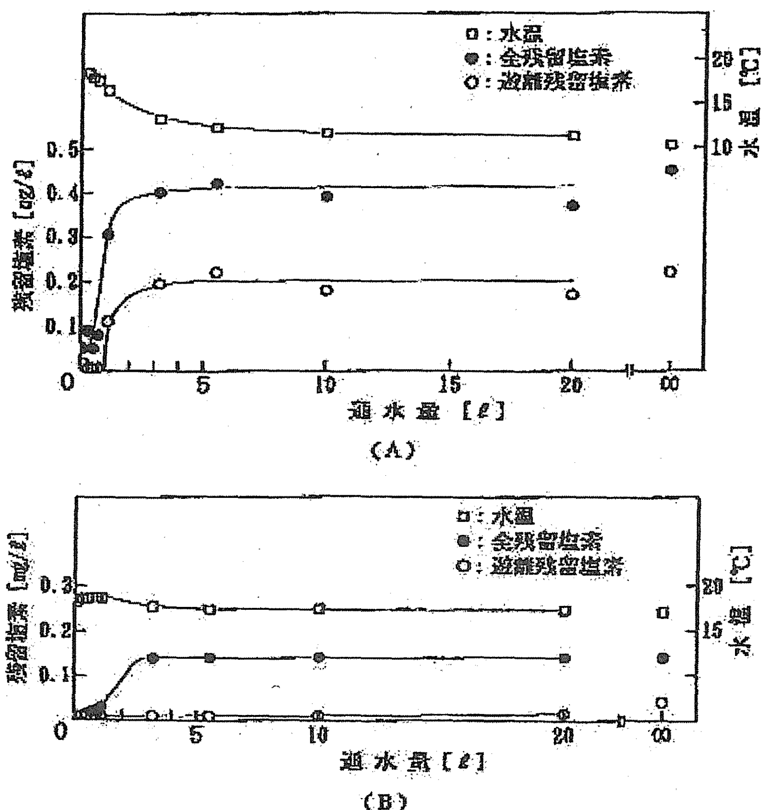

図1　残留塩素と水温の変動

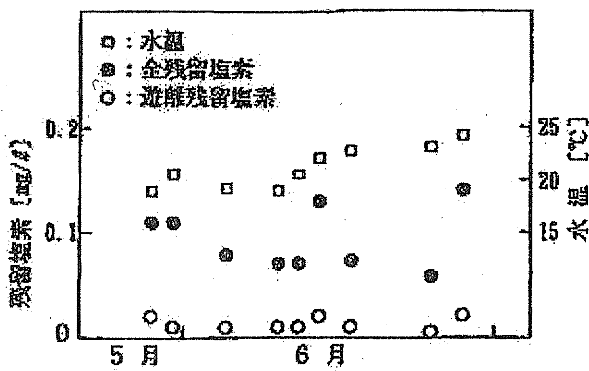

図2　5月・6月における残留塩素と水温

表1　残留塩素と水温

年	月	残留塩素[mg/ℓ] 遊離	全	水温[℃]
1996	7	0.00	0.03	28.2
1997	1	0.22	0.54	10.4
	4	0.03	0.14	17.0
	5	0.01	0.11	20.0
	6	0.01	0.10	23.7
	7	0.00	0.04	26.5

に低い値となっており一年のうちのかなりの期間において下限値をかなり下回っているという結果であった。同じ新宿区内の都庁付近の平成9年5月採水の水質検査結果では残留塩素が0.6mg/ℓ[7]であり、本学にも同レベルの水が配水されているはずであるが、給水栓からの水には大きな差異が生じている。安全性確保のためには供給水の遊離残留塩素濃度が0.2mg/ℓ以下の状態が発生することは好ましくない[8)9)]ということなので、今回の一連の残留塩素の測定値からいえば安心できない状況にあるようである。もちろん遊離残留塩素ゼロイコール細菌が増殖していると短絡的解釈は無謀であるが、増殖している可能性を否定できないことも確かなことである。当然のことながら、水道は施行規則の下限値より高濃度の塩素を含む水が受水槽に入ってくるわけだから、受水槽や高置水槽に貯留中に塩素が消費あるいは抜けてしまったということになる。消費は主に鉄サビや水アカによるが、一般的に言って管の内部や管と管との接合部に鉄サビが発生していることは確かなことである。揚水ポンプの水あげ口が底の方にありポンプ自体もさびていたり、鉄板製の高置水槽にサビが生じていることも否定できないところである。また、受水槽や高置水槽に水アカがたまっている可能性も否定できない。貯留中の塩素抜けもかなりの量に上るとみられる。特に高置水槽では高置水槽の水位が下がるとリレーの働きで揚水ポンプが作動し、受水槽の水が汲み上げられるようになっているが、水の使用量が少ない日が続けば受水槽からの水の流入はなく、気温の影響を直ちに受ける鉄板製の水槽に長い間同じ水が滞留しているわけである。受水槽も同様で、受水槽の容量に対して使用量が少なければ水が循環しきらずに滞留して塩素抜けが生じることになり、塩素を多く含んだ新しい水の流入も少なく、残留塩素濃度が低いままという結果になる。以上のことが複合的に作用して低残留塩素状態を生じていると考えられる。

結言

水道水中の遊離残留塩素と全残留塩素および水温の測定を行い、以下のことを明らかにした。

1) 残留塩素濃度は通水開始後約1ℓまでは低く、以降急激に変化し、3ℓ位通水すると長時間通水した後の濃度レベルに近くなる。
2) 残留塩素の水道施行規則の規定値を満たしたのは、冬季の測定の時のみで、春季の4月下旬では既に下限値の三分の一程度まで低くなっていた。一年のかなりの期間、残留塩素濃度は規定値よりはるかに低かった。

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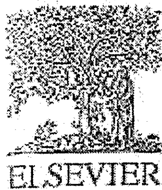
On the Variation of Residual Chlorine in Water

Toshikatsu TSUYUKI

An experimental study was made of variation of residual chlorine in water at T. W. M. C. School of Nursing from July, 1996 to July, 1997. Free and total residual chlorine were determined by means of chlorine colorimeter using DPD method.

The results were as follows :

1) Both the residual chlorine were low concentration till outflow of about one liter water, then varied greatly. After water had been flowed about three liter, the concentration of residual chlorine were nearly equal to that of water in the tank on roof of the scloolbuilding.
2) In January, the concentration of residual chlorine were higher than Regulations for the Enforcement of the Law of Water. In April, the residual chlorine were about a third of minimum value of the Regulations. On and after the latter part of May, free were below 0.01mg/ℓ and total were below 0.11mg/ℓ. Therefore both the residual chlorine are much lower than the Regulations for the major part of a year.



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Heterotrophic plate count bacteria—what is their significance in drinking water?☆

Martin J. Allen[a,*], Stephen C. Edberg[b], Donald J. Reasoner[c]

[a] Awwa Research Foundation, 6666 W. Quincy Avenue, Denver, CO 80235, USA
[b] Yale University School of Medicine, New Haven, CT, USA
[c] U.S. Environmental Protection Agency, Cincinnati, OH, USA

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Abstract

While the literature documents the universal occurrence of heterotrophic plate count (HPC) bacteria in soils, foods, air, and all sources of water, there is a lingering question as to whether this group of organisms may signal an increased health risk when elevated populations are present in drinking water. This paper reviews the relevant literature on HPC bacteria in drinking water, the lack of clinical evidence that elevated populations or specific genera within the HPC flora pose an increased health risk to any segment of the population, and the appropriate uses of HPC data as a tool to monitor drinking water quality changes following treatment. It finds no evidence to support health-based regulations of HPC concentrations.
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Keywords: HPC; Drinking water; Heterotrophic bacteria

1. Introduction

1.1. Terminology

The term "heterotrophic bacteria" includes all bacteria that use organic nutrients for growth. These bacteria are universally present in all types of water, food, soil, vegetation, and air. Under this broad definition, primary and secondary bacterial pathogens are included, as are coliforms (*Escherichia, Klebsiella, Enterobacter, Citrobacter, Serratia*).

Heterotrophic plate count (HPC) bacteria represent those microbes isolated by a particular method, whose variables include media composition, time of incubation, temperature of incubation, and means of medium inoculation.

Other terms that have been used to describe this group of bacteria in water include "standard plate count", "total viable count", "total count", "plate count", "total bacterial count", "water plate count", "colony count", "aerobic mesophilic viable count", and "autochthonous flora". All of these terms describe the same general bacterial group, i.e., the population of bacterial colonies produced on an agar-based medium under defined incubation temperature and time. With the 16th edition of *Standard Methods for the Examination of Water and Wastewater*, "Heterotrophic Plate

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* Corresponding author.

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Count" was the term selected to designate this group of bacteria in water.

It is important to understand that while the term "heterotrophic bacteria" denotes all bacteria requiring organic nutrients for growth, all HPC methods enumerate only a fraction or subpopulation of heterotrophic bacteria in any water, food, soil, vegetation, air, etc. Further, it is not possible to know which percentage of the subpopulation of heterotrophic bacteria is enumerated by any HPC method, and it is not possible to differentiate which of the subpopulation includes potential pathogens.

1.2. HPC media and methods

Through the years, many "standard methods" have been used to enumerate the very broad range of genera that comprise HPC populations in drinking water. Examples of such methods and their respective developmental histories are described in "Monitoring Heterotrophic Bacteria in Potable Water" (Reasoner, 1990).

Based on decades of research with a variety of HPC media and methods, the following observations have been made:

1. Although often referred to as non-selective media, all media used for HPC determinations, along with respective time and temperature conditions, are "selective" for those bacteria that can grow under those specific conditions.
2. There is no single medium or method that will recover or enumerate all bacteria in the water being analyzed.
3. Many heterotrophic bacteria that are present in water are not culturable at present.
4. The choice of culture medium, temperature, and incubation time are important with regard to HPC results from a given water sample.
   - Both high-nutrient and low-nutrient media are used for HPC determinations.
   - High-nutrient media are better for enumeration of bacteria from animals and humans.
   - Low-nutrient media are better for enumeration of water-based bacteria (autochthonous) found in aquatic systems, including drinking water. The most commonly employed heterotrophic medium is R2A. It was designed specifically as a low-nutrient, low-ionic strength formulation to isolate bacteria that have a water-based, rather than mammalian lifestyle (Reasoner, 1990).
   - New methods that employ fluorescent substrates have been developed (Jackson et al., 2000). Fluorescence permits more rapid results and has the potential for automation.
5. Time and temperature of incubation are very significant variables. Table 1 presents examples of variable differences on the resulting cfu/ml (Reasoner, 1990).
   - High-temperature incubation (35–37 °C) and short incubation time (34–48 h) favor the growth of bacteria from animals and humans.
   - Low-temperature incubation (20–28 °C) and longer incubation time (5–7 days) favor the growth of water-based bacteria.
6. All bacterial pathogens and opportunistic pathogens are heterotrophic bacteria, some of which can grow on media used for determining standard plate counts or heteroptrohic plate counts in drinking water. However, it is necessary to use selective or

Table 1
Comparison of HPC results using different media (adapted from Reasoner, 1990)

Temperature (°C)	Method (cfu/ml)		
	PP	SP	MF
35	3137 (SPC)	–	4273 (m-HPC)
20	170 (SPC)	440 (R2A)	510 (m-HPC)
20	–	4000 (R2A)	12 (m-HPC)
	–	1000 (R2A)	110 (m-HPC)
35	–	20 (R2A)	6 (m-HPC)
	–	4 (R2A)	<1 (m-HPC)
35	277 (SPC)	–	283 (m-HPC)
20	1123 (NA)	–	1217 (m-HPC)
	1192 (NA)	–	1192 (R2A)
35	22 (SPC)	200 (R2A)	32 (m-HPC)
28	80 (SPC)	360 (R2A)	140 (m-HPC)
20	22 (SPC)	90 (R2A)	47 (m-HPC)
35	53 (SPC)	–	66.7 (m-HPC)
	53 (SPC)	–	57.1 (R2A)
26	590 (SPC)	1550 (R2A)	–
22	100 (YEA)	710 (YEA)	–
	440 (R2A)		–
	100 (YEA)	3900 (R2A)	–

cfu, colony-forming units.
Media: SPC = standard plate count agar; NA = nutrient agar; YEA = yeast extract agar; R2A medium; m-HPC medium; m-HPC was published originally as m-SPC medium.

differential media to distinguish pathogens and opportunistic pathogens from non-pathogens.

7. There are differences between pour-plate, spread-plate, and membrane filtration methods.
   - The pour plate method generally yields lower bacterial counts regardless of medium or time of incubation and is generally limited to 0.1 to 1.0 ml sample volume.
   - The spread plate method generally yields higher counts than other methods but is limited to a 0.1 to 1.0 ml sample volume.
   - The membrane filtration method is more flexible because it allows for the analysis of sample volumes greater than 1.0 ml.
   - Different methods and media will produce markedly different HPC concentrations (see Table 1).

2. Heterotrophic bacteria genera

As described above, the genera enumerated by any HPC method are highly variable since the cultivation medium of choice, incubation temperature, incubation time, origin (river, surface water reservoir, treated and disinfected drinking water, etc.), season of the year, and age of the water sample have a significant effect on which genera will grow under these selected conditions. This same variability applies to the analysis of food, dairy, and other environmental determinations.

As mentioned above, the bacteria that fit the scientific definition of heterotrophic bacteria (use of organic nutrients as the energy source) include *Mycobacterium avium* complex and Legionella and may not grow on HPC media. Accordingly, if one wishes to determine if these genera are present in drinking water, specific methods tailored to their specific growth requirements must be employed.

2.1. HPC genera found in drinking water

There is a significant body of information in the literature on genera that comprise HPC populations enumerated in drinking water using different methods. Table 2 includes genera often reported in the literature (LeChavallier et al., 1980; Herson and Victoreen, 1980; Briganti and Wacker, 1995).

Table 2
HPC genera commonly found in drinking water

Acinetobacter	*Methylomonas*
Actinomycetes[a]	*Micrococcus*
Alcaligenes	*Mycobacterium*[a]
Aeromonas[a]	*Morexella*
Aeromonas hydrophila[a]	*Nitrobacter*
Arthrobacter	*Nitrosomonas*
Bacillus	*Nocardia*[a]
Beggiatoa	*Proteus*
Citrobacter freundi	*Pseudomonas*
Corynebacterium	*P. cepacia*
Crenothrix	*P. fluorescens*
Desulfovibrio	*P. maltophilia*
Enterobacter agglomerans	*Serratia liquefaciens*
Enterobacter cloacae	*Sphaerotilus*
Escherichia coli	*Sphingomonas*
Flavobacterium	*Staphylococcus*
Flavobacterium meningosepticum	*Streptococcus*
Gallionella	*Streptomyces*
Hafnia alvei	*Yersinia enterocolitica*
Klebsiella pneumoniae	

In addition to the above genera, HPC populations from drinking water include many pigmented (orange, yellow, pink) organisms that are difficult to speciate.

[a] Generally not recovered by HPC methods as referenced in *Standard Methods for the Examination of Water and Wastewater*, 20th edition.

2.2. HPC genera found in food

All of the HPC genera found in drinking water are also common in foods, and humans ingest large numbers of these microorganisms daily. While the upper range of HPC populations in drinking water average 5000–10,000 cfu/ml (Reasoner, 1990), HPC populations in food are consistently log concentrations higher (Wadhwa et al., in press). This difference is because food provides distinctly different physical and physiological conditions than drinking water. With higher concentrations of carbohydrates, protein, and ionic strength, food is much closer to the human physiological state than drinking water, which is essentially devoid of nutrients and ionic strength. Accordingly, microbes that can multiply in humans and cause disease can grow in foods but do not multiply in drinking water. Virtually all foods contain many thousands times more bacteria than drinking water. Table 3 provides examples of microbial genera and densities found in foods.

Wadhwa et al. (in press) concluded, based on both observed microbial content and the potential presence

Table 3
Examples of microbial indicators and densities found in food (Wadhwa, 2002)

Indicator/genus	Food	Density (cfu/g)
Aerobic plate count	ground beef	8×10^3
Aerobic mesophilic bacteria	retail white cabbage	10^6
	retail lettuce	10^6
	retail cucumber	10^5
	retail green pepper	10^4
Aeromonas spp.	green leaf lettuce	7.3×10^3
	spinach	5.1×10^3
Aerobic plate count	packaged carrot sticks	10^6

of large numbers of pathogens or their indicators in food, that food is more of a health risk than drinking water. They also stated that naturally occurring bacteria (HPC or autochthonous flora) do not have virulence factors, making their numbers in drinking water irrelevant to health risk except in the most severely immunocompromised subpopulations, who are fully aware of their medical condition and need to exercise appropriate dietary and other preventive measures. (Edberg, 1997).

3. Association of health risk and HPC bacteria

As noted earlier, the broad definition of HPC bacteria includes a wide range of bacterial genera, which may include primary and secondary pathogens. Specific HPC genera that some consider as opportunistic pathogens enumerated by HPC methods include *Aeromonas*, *Klebsiella*, and *Pseudomonas*. Because of this fact, regulatory health agencies and some microbiologists suggested that HPC bacteria be considered a health-based drinking water parameter. In 1984, the U.S. Environmental Protection Agency (EPA), Office of Drinking Water, drafted the document "Drinking Water Criteria Document on Heterotrophic Bacteria"(unpublished). EPA did not move forward on promulgating an HPC-based regulation because there was insufficient clinical evidence that the addition of a maximum limit on HPC populations would provide a higher level of public health protection than that afforded by existing regulations (Reasoner, personal communication).

The U.S. Food and Drug Administration (FDA) examined the possible health effects of HPC concentrations in regards to bottled water. The FDA first examined the role of HPC human disease in 1973 and failed to issue a regulation. In 1993, the FDA reviewed the subject again in detail and wrote: "FDA still believes that, when bottled waters are free of microorganisms that are of public health significance (i.e., indicated by the absence of coliforms) and are bottled under sanitary conditions in compliance with good manufacturing practices (CGMP) regulations, the presence of heterotrophic bacteria that are part of the flora in those bottled waters normally will not pose a health risk because these organisms do not colonize the digestive tract of humans" (Federal Register, 1993).

The question remains, since the 1984 EPA draft document, whether there is more recent and compelling clinically based information to consider HPC bacteria as an indicator of increased health risk. Several epidemiological studies (Calderon, 1988, 1991; Payment et al., 1991) examined the concentration of HPC bacteria in drinking water and possible health effects. In a point-of-use study (Calderon and Mood, 1988) and a point-of-entry study (Calderon and Mood, 1991), no association between adverse health and HPC was noted. Payment et al. (1991) found that there was an association between HPC and gastroenteritis but not between the amount of drinking water and disease. Colford (2002) suggests that this anomaly may have been the result of the lack of double blinding (i.e., each consumer knew which group they were a member of—drinking water treated or not treated) of the study. A more recent Australian epidemiological study (Hellard et al., 2001) found no clinical correlation with elevated HPC populations in drinking water, but earlier studies yielded equivocal results.

3.1. Cytotoxicity and invasiveness of HPC bacteria

For a heterotrophic bacterium to pose a health risk when consumed in drinking water, it must be present at an infectious dose (i.e., sufficient concentrations) and be capable of infecting a human host. The capability of a microorganism to cause disease is often referred to as virulence. Frank or primary pathogens possess a wide range of virulence factors, which enable them to circumvent human defense mechanisms (Duncan and Edberg, 1995). Because epidemiological and animal infectivity studies are complex, difficult to control, expensive, and yield

circumstantial evidence, Edberg et al. (1997) directly examined HPC bacteria with regard to cytotoxicity and invasiveness factors that directly relate to the probability of a microorganisms successfully causing disease.

HPC bacteria in tap water and bottled water were enumerated using R2A medium. R2A isolates were subsequently inoculated onto 5% sheep blood agar, since it is physiologically equivalent to the human condition and HPC bacteria capable of causing infection (possessing virulence factors) should grow on blood agar. Those R2A isolates that did grow on blood agar were inoculated into the human colonic adenocarcinoma cell line (CACO-2_{BBe} ($C2_{BBe}$) to determine their invasiveness. With respect to cytotoxicity, the cfu/ml counts on blood agar were between 10^{-2} and 10^{-3} less on blood agar than found on R2A agar. Of the 85 R2A isolates that grew on blood agar, only 10 demonstrated invasiveness. The bacteria isolated in this study did not possess significant virulence characteristics associated with a human health threat.

Other investigators (Lye and Dufour, 1991; Payment et al., 1994; Edberg, 1996; Edberg et al., 1996) reported similar results, i.e., few virulence factors such as α, β, γ hemolysis, adherence, and invasiveness. Smith et al. (2001) examined the ability of HPC to express virulence in highly immunosuppressed mice and found these factors lacking.

3.2. Opportunistic pathogens

Certain heterotrophic bacteria are considered opportunistic pathogens, i.e., capable of causing disease only in compromised human hosts. Several of these microorganisms can be found in source waters and in treated drinking water and can be enumerated on HPC media. Microorganisms most often called opportunistic pathogens in drinking water include *Pseudomonas*, *Klebisella*, and *Aeromonas*. It is important to understand that the basis for these genera being opportunistic pathogens is associated entirely with nosocomial (hospital-acquired) infections, not ingestion from consumption of drinking water. In hospitals, the route of transmission is not drinking water but medical devices. Duncan (1988) provides an excellent review of this difference and can be used as a model for other potential opportunistic pathogens that may be found in drinking water.

The determination that a specific microorganism is truly an opportunistic pathogen associated with drinking water and the decision by public health agencies to regulate specific microorganisms that may be found in drinking water can only be made if specific criteria indicate that a microorganism poses a health risk.

These criteria include the following:

1. There is a clinical history of an organism causing disease from ingestion of drinking water.
2. There is epidemiological evidence that drinking water, rather than food or other vectors, is a major source of disease.
3. There is sufficient evidence that the target organism, i.e., opportunistic pathogen, is found in water in sufficient concentrations and possesses virulence factors capable of causing disease in humans.
4. There is sufficient evidence that the target organism is not readily removed or inactivated by conventional water treatment processes (coagulation–filtration–disinfection).
5. There is sufficient evidence that the target organism, if surviving conventional treatment, will be viable, virulent, and present in sufficient numbers to cause disease.
6. There are robust analytical methods for the target organism, which have acceptable sensitivity, specificity, and reproducibility to accurately measure the presence of the target organism in treated drinking water.
7. The performance criteria of analytical method(s) for the target organism have been certified by the public health agency, and there is intra-laboratory performance on which to base this certification.
8. There is sufficient evidence that the target organism is present in high concentrations in these same waters.

On the basis of these criteria, *Klebsiella*, *Pseudomonas*, and *Aeromonas* should not be considered opportunistic pathogens in drinking water.

3.2.1. Klebsiella

While the genus *Klebsiella* is enumerated by HPC methods and is a coliform, it does not fulfill the criteria noted above and should not be considered an opportunistic pathogen from drinking wa-

ter. In a review of waterborne *Klebsiella* and human disease (Duncan, 1988), the author stated the following:

> *Klebsiella* occurs widely in nature and is often present in surface water used for human consumption or for recreational purposes. The organism can survive in water distribution systems despite chlorination. Many strains give rise to positive fecal coliform tests, even when they are the only organisms present in the water sample. The public health significance of *Klebsiella* in water is therefore an important concern. In the past, *Klebsiella* was thought to be a significant pathogen in the community causing serious primary pneumonia, but such cases are now extremely rare. Serious *Klebsiella* infections are today commonly seen only in hospital patients whose resistance has been impaired by their primary disease condition. There is no evidence that waterborne *Klebsiella* play any significant part in the epidemiology of these hospital-acquired infections. *Klebsiella* in water supplies should therefore not be considered a hazard to human health.

Duncan's analysis of *Klebsiella* applies to other potential opportunistic drinking water pathogens.

3.2.2. Pseudomonas

The genus *Psuedomonas* is also routinely enumerated in HPC determinations and considered by some to be an opportunistic pathogen when found in drinking water. In a 1997 review, "*Pseudomonas aeruginosa*: Assessment of Risk from Drinking Water" (Hardalo and Edberg, 1997) analyzed all reports of this bacterium as a gastrointestinal pathogen and concluded:

> *Pseudomonas aeruginosa* is an ubiquitous environmental bacterium. It can be recovered, often in high numbers, in common food, especially vegetables. Moreover, it can be recovered in low numbers in drinking water. A small percentage of clones of *P. aeruginosa* possess the required number of virulence factors to cause infection. However, *P. aeruginosa* will not proliferate on normal tissue but requires previously damaged organs. Further narrowing the risk to human health is that only certain specific hosts are at risk, including patients with profound netropenia, cystic fibrosis, severe burns, and those subject to foreign device installation. Other than these very well defined groups, the general population is refractory to infection. Because of its ubiquitous nature, it is not practical to eliminate *P. aeruginosa* from our food and drinking water, but attempts to do so would produce disinfection byproducts more hazardous than the species itself. Moreover, because there is no readily available sensitive and specific means to detect and identify *P. aeruginosa* available in the field, any potential regulation governing its control would not have a defined laboratory test measure of outcome. Accordingly, attempts to regulate *P. aeruginosa* in drinking water would not yield public health protection benefits and could, in fact, be counterproductive in this regard.

3.2.3. Aeromonas

Aeromonas is another genus naturally found in drinking water. It may or may not be isolated on HPC media (Payment et al., 1994) methods and has been suggested as an opportunistic pathogen when present in drinking water. Similar to the above analysis as to why neither *Klebsiella* nor *Pseudomonas* is an opportunistic pathogen when present in drinking water, *Aeromonas* also fails to meet most, if not all, of the above criteria as a gastrointestinal pathogen by ingestion.

In a review paper by Edberg and Allen (in preparation) entitled "Issues for Microbial Regulations: *Aeromonas* as a Model," the authors provide data to make the following observations:

1. A small percentage of *Aeromonas hydrophila* isolates can cause gastroenteritis and enteritis and produce modest, self-limited infection. Although most cases are food-borne, the few waterborne cases were associated with ingestion of untreated drinking water from shallow wells. These waters are also very high in assimilable organic carbon concentrations.
2. The concentration of *A. hydrophila* from food is much higher, by several logs, when compared to water sources. The species *A. hydrophila* is the most important. Only a small percentage of *A.*

hydrophila isolates possess human virulence factors.

3. There is little overall similarity between diarrheal and water isolates. Water isolates that can infect humans are rare.
4. There are many species of *Aeromonas*. Based on isolates from cases of gastroenteritis, *A. hydrophila* is the only one associated with human disease. Even within the species *Hydrophila*, only a small percentage of isolates produce sufficient virulence factors to cause disease. Accordingly, laboratory tests for *Aeromonas* must not only be specific for the species but also the virulence factors.
5. *Aeromonas*, including *A. hydrophila*, are of low virulence. Animal studies show it requires large numbers of bacteria inoculated intraperitoneally to cause disease. Human feeding studies with ingestion of 10^8 cells have not produced disease.
6. No *Aeromonas* medium that has acceptable sensitivity, specificity, and reproducibility for the detection of *A. hydrophila* has been developed or used extensively for drinking water. There has not been widespread use or testing of media for this bacteria from drinking water.
7. *Aeromonas* isolates exhibit no exceptional resistance to chlorine disinfection at concentrations and exposure times typically found in public water systems.

Based on these observations, there is insufficient evidence that *A. hydrophila* can be considered an opportunistic pathogen when present in drinking water, and it would be inappropriate to consider monitoring or regulating this organism at this time.

4. Significance of HPC populations in drinking water

As mentioned previously, the number of HPC bacteria in drinking water varies widely. It depends on the quality of the source water, the types and efficacy of treatment, the type and concentration of disinfection residuals, the age and the condition of the storage and distribution system, the concentration of dissolved organics in the treated drinking water, the ambient temperature of the raw and finished water, the elapsed time between the water treatment plant and sampling locations, and, of course, the HPC method and time and temperature of incubation. These are just some examples of variables that have a profound effect on the enumeration of HPC bacteria. With all these of variables, it is obvious that the range of HPC populations in drinking water is considerable, i.e., <0.02 to 10^4 cfu/ml or higher.

While there is a lack of health-based justification for setting an upper HPC limit in drinking water, a number of countries have established mandatory limits for HPC bacteria in drinking water. As would be expected, different countries use a variety of terms to describe their respective bacterial count method, specify different analytical procedures (media, temperature, time) that can be used, and establish different maximum acceptable counts, which can range from 20 to 1000 cfu/ml. Some have argued that lower HPC bacterial populations in drinking water are more desirable than higher populations, but there is no epidemiological evidence that higher HPC populations have any public health significance. Typically, public water systems with conventional treatment are able to limit HPC bacterial populations to below 100 cfu/ml in the distribution system, although many systems experience increased HPC populations (500–1000 cfu/ml) during the summer months. Bottled water that has no disinfectant residual may have much higher HPC populations. While a maximum HPC population of 500 cfu/ml in drinking water is often cited as a health-based standard, this perception is fallacious and not based on fact. As reviewed below, there is no health-based substantiation for HPC regulations.

4.1. Origin of basis for establishing maximum HPC populations

The commonly used "level of concern", 500 cfu/ml, originated from studies that examined the effect of HPC populations on analytical recovery of total coliforms. It was never a health-based action level.

Possibly the first evidence that high HPC populations may interfere with the detection of coliforms by the multiple-tube-fermentation method (MTF) or the membrane-filtration method (MF) was suggested by McCabe et al. (1970). In reviewing the bacteriological results from a 1969 survey of 969 public water systems in the US, the authors stated: "While bacteria

enumerated by plate count do not usually have a direct health significance, heavy growths of bacteria and other microorganisms do indicate the potential for contamination. Also, research findings (Geldreich, 1972) suggest that high plate counts inhibit the growth of coliform bacteria on laboratory media, thereby obscuring their presence". They further examined the question of interference specifically and reported that the 1969 survey data found the frequency of detecting total and fecal coliforms by the membrane-filtration method increased as the SPC levels increased to 500 cfu/ml, but decreased in frequency when SPC levels exceeded 1000 cfu/ml.

To further examine this interference phenomenon, Geldreich et al. (1978) collected 613 samples from 32 dead-end water main flushing sites in the Cincinnati, OH, distribution system. This study found 76 samples contained coliforms by the MTF procedure, but only 19 by the MF procedure. Data analysis demonstrated a correlation between excess SPC densities and desensitization of the MF method when SPC bacteria exceeded 500 cfu/ml. Other researchers (Clark, 1980; Herson and Victoreen, 1980; Means and Olson, 1981; Seidler et al., 1981; Burlingame et al., 1984; Franzblau et al., 1984) have also reported method desensitization or coliform antagonism by HPC bacteria clustering in the 500–1000 cfu/ml range. These investigations demonstrated that high SPC (HPC) densities can substantially interfere with both the MTF method and especially the MF method, but that this phenomenon may not occur consistently.

One of the co-authors of the 1978 report is also a co-author here. From the original analytical data demonstrating interferences by HPC on the recovery of coliforms, to 25 years later, the following have been demonstrated:

1. There is no EPA, FDA, or WHO health-based HPC regulation.
2. HPC concentrations are mentioned only twice in EPA regulations: first, as a cause of false-negative coliform tests in which lactose-based media (i.e., MTF and MF) are employed and second, as a surrogate for chlorine residuals in distribution systems.
3. Suppression of coliform recovery only occurs with lactose-based media formulations. Defined Substrate Technology methods (e.g., Colilert®, Colisure®) do not suffer from HPC suppression.

4.2. Significance and impact of HPC bacteria on coliform detection methodology

The ramifications of HPC populations greater than 500 cfu/ml in drinking water are significant because they desensitize membrane-based coliform methods that contain lactose. Given that routine analysis of drinking water for coliforms and *Escherichia coli* is the most common and the most important determination as to the microbiological safety of drinking water, desensitization by HPC bacteria may have grave public health consequences. For this reason, it is imperative that HPC analysis be performed in parallel with each MF coliform/*E.coli* determination. This quality assurance approach ensures that coliform/*E. coli* data, especially negative results, accurately reflect the true microbiological quality of drinking water.

In the late 1980s, the development of the Defined Substrate Technology (Edberg et al., 1988) for the simultaneous enumeration of coliforms and *E.coli* provided a method that was not subject to HPC interferences, resulting in greater confidence that negative coliform/*E.coli* drinking water samples correctly reflect their microbiological quality.

5. Uses of heterotrophic plate count measurements

While there is no validated clinical evidence that the consumption of drinking water containing high levels of HPC bacteria poses increased health risks, HPC measurements do have value as a tool to ensure drinking water quality. The purpose of water treatment is to provide a safe water supply through the use of unit processes that reduce turbidity, and chemical, and microbiological contaminants to desired levels. Beyond the water quality gains as a result of treatment, there remains the challenge of maintaining water quality during storage and distribution prior to reaching consumers.

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 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 after growth potential in treated drinking water;
6. monitoring bacterial population changes following treatment modifications such as a change in the type of disinfectant used.

6. Summary

1. While the literature documents the universal occurrence of HPC bacteria or autochthonous flora in soil, food, air, and all types of water, there is insufficient clinical and epidemiological evidence to conclude that HPC bacteria in drinking water pose a health risk. For this reason, it is not possible to establish health-based standards for HPC bacteria in drinking water.
2. The various methods used to enumerate HPC bacteria differ significantly in the number and genera detected, and HPC data from different methods are not necessarily comparable.
3. HPC populations greater that 500–1000 cfu/ml in drinking water can interfere with coliform/*E. coli* analysis by lactose-based methods, which include the membrane-filtration method.
4. *Klebsiella*, *Pseudomonas*, and *Aeromonas* cannot be considered opportunistic pathogens when found in drinking water, since there is no clinical or epidemiological evidence to support this designation.
5. HPC determinations can be a useful tool to the monitor efficacy of drinking water treatment processes and undesirable changes in bacterial water quality during storage and distribution, but not because of health-risk reasons.

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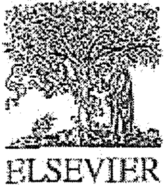

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Heterotrophic plate count monitoring of treated drinking water in the UK: a useful operational tool[☆]

David P. Sartory*

Quality and Environmental Services, Severn Trent Water, Welshpool Road, Shelton, Shrewsbury SY3 8BJ, UK

Abstract

The count of general or heterotrophic bacterial populations in treated drinking water in the UK has been undertaken since the 1880s. Counts of heterotrophic bacteria at 22 and 37 °C are used widely as part of an overall assessment of treated drinking water quality. There were no legislated standards for water quality in the UK until adoption of the first EU Directive in 1989. The UK has, however, never stipulated numerical standards or guidelines for heterotrophic bacteria, although their enumeration has long been part of the assessment of 'wholesome' water, on which advice regarding microbiological quality was given in a series of documents known as 'Report 71'. The current regulations stipulate only that there should be 'no abnormal change' in numbers normally associated with a given supply. This paper reviews the historical context regarding the enumeration, and interpretation of results, of heterotrophic bacteria from treated drinking water, and information regarding current practices by UK water suppliers. The appropriateness of using heterotrophic bacteria counts as an operational tool or as a health parameter is briefly discussed in the light of the UK experience.
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Keywords: Heterotrophic bacteria; Plate counts; Drinking water; UK standards

1. The use of heterotrophic plate counts in the UK (1885–2002)

In the summer of 1884, Percy Faraday Frankland visited the International Health Exhibition at South Kensington in London, and saw a demonstration of the technique for the cultivation of bacteria on solid nutrient gelatine medium that Robert Koch had been developing over the previous 5 years. A lengthy description of the method was also published in the *Lancet* (Hamlin, 1990). Frankland, who was a chemist by training and had an interest in the quality of water being supplied to London, appreciated that the technique could be applied to culturing and counting bacteria from untreated and treated water, and together with his wife, Grace, adapted the method. One millilitre of water was added to a molten mixture of gelatine and filtered meat broth and incubated at 18–22 °C (basically at room temperature) for a few days, counting colonies as they developed (Frankland and Frankland, 1894). At the beginning of 1885, Frankland started testing various waters supplied to London and, in the

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* Tel.: +44-1743-265765; fax: +44-1743-265043.
E-mail address: david.sartory@severntrent.co.uk (D.P. Sartory).

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process, inaugurated the first monthly systematic bacteriological examination of the supplies (Garner, 1948). The results of this testing were soon to be published by the then official 'Water Examiner' Colonel Francis Bolton. In mid-1885, Frankland also applied the technique to testing water before and after sand filtration, employed by some London water companies, recording reductions in numbers of bacteria of greater than 88%, and to testing the effectiveness of different filter materials (Frankland and Frankland, 1894). With this work, the counting of general bacterial populations (subsequently termed plate counts, then colony counts, and now heterotrophic plate counts (HPC)) in drinking water became firmly established as an important parameter for the assessment of water quality.

The use of plate counts became widely used and an incubation temperature of 18–22 °C became the norm with daily examination of plates for up to 5 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 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 recognised that many other naturally occurring bacteria were also capable of growing at this temperature (Savage, 1906). During this time, counts at 18–22 or 20–22 °C were typically conducted using nutrient gelatine plates, and those at 37 °C using nutrient agar plates (Royal Institute of Public Health, 1904; Savage, 1906). Formal guidance on the bacteriological examination of water supplies and the interpretation of results was first published by the UK Ministry of Health in 1934 (Anon., 1934) as what was to become universally known as 'Report 71'. The recommended method involved dispensing of 1 ml aliquots of water, mixing with nutrient agar and incubation of one set of plates at 20–22 °C for 3 days and another set at 37 °C for 2 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' whilst 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' (Anon., 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' (Anon., 1934). This approach was reaffirmed in the second edition of 'Report 71', published 5 years later (Anon., 1939).

Experience gained over the next 17 years, however, led to a change of emphasis in the third edition (Anon., 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 that 'a rising plate count may give the earliest sign of pollution' (e.g. in wells) (Anon., 1956). This interpretation of the value of plate counts was reiterated in the fourth and fifth editions of 'Report 71' (Anon., 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 (YEA) 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' (Anon., 1982). All reference to potentially indicating faecal contamination had been dropped.

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 recognised (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 recognised 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 microbiological 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,b) prepared with regard to the new UK legislation (Anon., 2000) arising from the 1998 EU Directive (European Union, 1998). The guidance re-emphasises 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 UK 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). 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. This edition also re-introduced 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 ISO standard ISO 6222:1999 (International Organisation for Standardization, 1999) stipulated by the 1998 EU Directive (European Union, 1998) as the method to be used. The lower 22 °C incubation range in the ISO standard is 22 ± 2 °C, which is a wider range than the 20–22 °C historically used in the UK and recommended by the UK guidance (Standing Committee of Analysts, 2002b).

The use of counts of heterotrophic bacteria has, therefore, a long history in the UK. 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 UK, despite being dropped in the EU Directive.

2. Current use of heterotrophic plate counts in the UK

There are 35 public suppliers of water in the UK (Table 1), all of whom participated in a survey regarding the monitoring of HPC populations. The suppliers were asked for information regarding their HPC monitoring under the UK regulations and for nonregulated operational purposes (e.g. customer complaint and allied investigations, new mains and rehabilitation work acceptance).

2.1. Use of data from regulatory monitoring

The UK regulations (Anon., 2000) stipulate frequencies for analysis of plate counts from treatment works final waters, service reservoirs and water supply zones. These are as follows:

(a) Water treatment works—depending upon the size of works (in terms of volume supplied) these range from daily (large works) to weekly (small works),
(b) Service reservoirs—weekly,
(c) Water supply zones—depending upon the population served; these range from twice a week to monthly.

Reduced frequencies of analyses can be applied to water treatment works and water supply zones depending upon previous results.

The principal use of the data gathered from regulatory monitoring is to monitor trends or deterioration (in terms of rising counts) in quality. Many suppliers targeted trend monitoring with data from service reservoirs. Other uses of the data included:

(a) chlorine management,
(b) microbial populations modelling,
(c) treatment works performance assessment,
(d) assessment for planned maintenance of infrastructure (e.g. cleaning of service reservoirs), and
(e) secondary indicators of quality following isolation of coliforms or other primary indicators.

Table 1
Number of public water suppliers in UK by legislative region

England	24
Wales	2
Scotland	3
Northern Ireland	1
Channel Isles	3
Isle of Man	1
Isles of Scilly	1
Total	35

Many suppliers reviewed plate count data for a supply area whenever an unusual result was recorded. Most suppliers also had regular review periods, typically monthly, half yearly or annually, some undertaking reviews on both a regular basis and by an unusual result. Most suppliers undertake these reviews on an informal basis, but several have a formal program, some linked in with their Quality Assurance procedures (e.g. ISO 9002, International Organisation for Standardization, 1994).

2.2. Use of plate counts in operational monitoring

All bar two suppliers undertake plate count testing for various areas of operational monitoring (Table 2). 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 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, and not that HPC bacteria may be related to the 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.

2.3. What is an 'abnormal change' in plate counts?

When the UK adopted the first European 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' (Anon., 1989a). Guidance from the regulators

Table 2
Use of plate counts for operational monitoring by 33 UK suppliers

Operational activity	Number of suppliers conducting plate counts at	
	37 °C	22 °C
Acceptance of new mains	23	21
Testing after rehabilitation work	23	20
Taste and odour investigations	26	25
Health complaint investigations	33	31
Biofilm investigations	14	15
Following repair of mains	8	5
Other investigations	9	8
Raw water monitoring	4	1

(Anon., 1989b) stated that 'continuous review is needed of colony counts' and 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 UK regulations (Anon., 2000) do not set numerical standards or guideline values for the 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 'what is an abnormal change?'. There is, currently, no official guidance on this in the UK (or Europe) and, consequently, there are several approaches that have been adopted by the water suppliers (Table 3).

Many suppliers employ simple numerical values for an indication of an abnormal change in counts from regulatory samples, some based on the guideline values of the first EU Directive (European Union, 1980), whilst some have adopted higher values (Table 3). These values generally serve as triggers to review previous data and make an assessment of any significance of the increase. Several factors appertain to the use of these numerical values, such as type of source (pristine groundwater sourced supplies generally have lower values allocated than lowland river sourced supplies), seasonal variation and local knowledge of water quality behaviour in the supply area. In practice, since counts of heterotrophic bacteria in UK public water supplies are generally low, counts exceeding these limits would represent a marked rise in bacterial populations. Several suppliers, however, have established arbitrary levels of increase ranging from 0.5 to >2 log increases over previous results (Table 3). 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 to 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 year or more. Generally, those that are derived for groundwater sourced supplies can be calculated from annual or more data as the counts tend to be more stable in the less biologically active water. When applied to more nutrient-rich surface water derived (and thus more variable) supplies, shorter data periods would tend to be used.

Table 3
Definitions of 'abnormal change' and operational limits for plate counts used by UK suppliers

Type of definition	37 °C	22 °C
Numerical limits for regulatory samples (cfu/ml)	>10, >20, >50, >100, >200, >300, >500	>50, >100, >300, >500
Numerical change in counts for regulatory samples	>0.5 log, >1 log, >2 log, >2.3 log	
Statistical change in counts for regulatory samples	(a) >3 standard deviation from previous six results	
	(b) 20 times 3-year mean	
	(c) >1.5 times 12-month rolling mean	
	(d) >98 percentile of rolling annual mean (22 °C)	
Numerical limits for operational samples (cfu/ml)	>10, >50, >100, >300, >500, >1000	>50, >100, >300, >500, >1000