Assessment of pharmaceutical water systems

10

10.1 Introduction

Water and the associated distribution systems can have a microbiological implications for both pharmaceuticals and healthcare [1]; this is especially so when water systems are poorly specified, improperly installed or not maintained to the appropriate standards. Microbiological risks are significant because water acts as a vector for microorganisms and it provides, with the addition of nutrients, the basis for microbial replication. Under most circumstances, the risks presented from water systems can be largely controlled by purification. This control is important for the use of water is, in pharmaceutical manufacturing, unavoidable.

Water is one of the most important materials within the pharmaceutical sector. Water is the basic ingredient of fermentation media, buffer manufacture, product extraction, purification, and as a solvent for dissolving products; furthermore, water is used for equipment cleaning, vial rinsing, diluting detergents, and so on [2]. While the quantities of water required will vary between facilities, one review estimated that 30,000 L of water is required to support the manufacture of 1 kg of a standard pharmaceutical [3]. Indeed the extensive use of water is one of the economic drivers for the adoption of single use, sterile disposable technologies. While disposable technologies can lead to economic savings through reducing the level of equipment washing and sterilization, the use of water as a pharmaceutical ingredient is inescapable.

Pharmaceutical facilities purify water through various treatments, culminating in either reverse osmosis or distillation. The objectives of water purification are threefold:

1. to reduce the levels of the chemical components in the water to prevent interactions with the drug substance, and to prevent toxicity;
2. to reduce the microbial bioburden to specified levels and to prevent further microbial proliferation;
3. to remove endotoxins and to prevent their future accumulation (this depends upon the grade of the water; not all grades of water are intended to the "endotoxin free").

The success of purification rests on the design and operation of the system. Due to the vagaries of these aspects, and based on the criticality of water in pharmaceutical production, water sampling and testing is subject to a high frequency of microbiological testing. This chapter examines the different types of water used in pharmaceutical facilities together with production methods. The chapter then proceeds to consider the microbiological risks and concludes with the main methods for microbiological monitoring.

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10.2 Pharmaceutical facility water

The different types of water found in pharmaceutical production plants are potable water, purified water, highly purified water, and water for injection (WFI).

1. Potable water (sometimes called towns water or mains water).

This is water of drinking water standard, provided to the pharmaceutical company via the municipal water supply. Potable water is used for the routine cleaning of less critical areas, as with the preparation of detergents and disinfectants. It is also the source water for the purification step required to manufacture pharmaceutical grade water (purified water and water-for-injections) [4].

Private water companies or municipalities will supply potable water according to the local quality requirements. These requirements are designed to protect human health. Health protection is concerned with ensuring that levels of chemical pollutants remain within established safety criteria, and so that water-borne diseases will not be transmitted (such as the parasitic *Cryptosporidium* and bacterial pathogens). The types of microorganisms screened for include the indicator organisms: *Escherichia coli*, enterococci, and *Pseudomonas aeruginosa*.

The monitoring standard almost universally applied is a heterotrophic plate count, with a limit of 500 colony forming unit (CFU) per milliliter or less, and the absence of indicator microorganisms of fecal origin in samples of 100 mL. While testing is mandatorily conducted by the water providers, some pharmaceutical facilities elect to carry out parallel testing of the water provided to the site. Many pharmaceutical companies hold mains water in storage tanks, and it is prudent to sample this water to ensure that there is not an increase to the microbial levels.

2. Purified water

Purified water is used as a solvent in the manufacture of aqueous and oral products, such as cough mixture, and for the generation of fermentation products. It is also used in the preparation of detergents and disinfectants for the cleaning of certified cleanrooms of EU good manufacturing practice (GMP) Grade C/ISO 14644 class 8 (in operation) and those areas of a lower classification. Purified water also acts as the source of the steam supply to autoclaves. This grade of water is additionally used for the final rinsing of equipment and as the ingredient water for nonsterile products.

Purified water is typically produced by reverse osmosis. Reverse osmosis units use a semipermeable membrane and a substantial pressure differential to drive the water through the membrane in order to achieve chemical improvements, and microbial and endotoxin reductions.

Reverse osmosis systems exist in multiple design formats. In general terms, reverse osmosis functions as a size-excluding filter operating under a highly pressurized condition. An effective system will block 99.5% of endotoxin as well as ions and salts, while allowing water molecules through. In removing endotoxin, the system acts as a molecular sieve through which lipopolysaccharide cannot pass. The reader should be aware that there is some debate as to the relative effectiveness of reverse osmosis compared with distillation.

The microbial action limit is 100 CFU/mL (equivalent to 10,000 CFU/100 mL, given that the recommended test method is by membrane filtration).

3. Highly purified water

This is a type of water described in the European Pharmacopoeia (Ph. Eur.). The specification is very similar to the specifications for purified water, with the main difference being a specification for endotoxin. This grade of water is intended for use in the preparation of medicines where water of high biological quality is needed. It is used for sterile medicinal products that are not required to be apyrogenic such as, ophthalmic, nasal/ear, and cutaneous preparations. The water is prepared by reverse osmosis, and the microbial action limit is 10 CFU/100 mL. The endotoxin specification is set at the same level as per WFI (0.25 EU/mL).

4. Water-for-injection (WFI).

WFI is the "purest" form of pharmaceutical grade water. WFI is used for the generation of microbial fermentation media and the preparation of culture media use for cell lines. The water is also used as a raw material in the manufacture of pharmaceuticals intended to be sterile, and for the preparation of detergents and disinfectants used in higher grade cleanrooms, such as EU GMP Grade B/ISO 14644 class 7 (in operation) areas. Where water is required to reconstitute vials of lyophilized product, sterile WFI is provided (i.e., WFI that has been subject to a terminal sterilization process).

With WFI, the specifications of the Ph. Eur. and United States Pharmacopeia (USP) are very similar. However, there is a fundamental difference in opinion concerning its preparation. In the United States, WFI may be prepared either by reverse osmosis or by distillation, whereas the European authorities insist that only distillation be used for its production (there is, at the time of writing, some debate as to whether there will be harmonization with the US approach thereby permitting the use reverse osmosis. This does not have scientific consensus, and the main concern with reverse osmosis centers on the risk of endotoxin build-up).

Distillation functions by turning water from a liquid to a vapor and then from vapor back to liquid. Through this process, endotoxin is removed by the rapid boiling activity. This causes the water molecules to evaporate and the relatively larger lipopolysaccharide molecules to remain behind. Most models of distillation equipment are validated to achieve 2.5–3 log reductions in endotoxin concentration during distillation. This is based on lipopolysaccharide having a molecular weight of around 10^6 Da. Hence, endotoxin is heavy enough to be left behind when water is rapidly boiled off as in a still.

With the operation of distillation units, the principal concern is with the entrainment of contamination, particularly endotoxin. Low levels of Gram-negative microorganisms in the feed water will contribute endotoxin, which are concentrated by evaporation. In poorly designed or maintained systems, levels of endotoxin build-up can occur in the reservoir of the still.

To meet the requirements of the Ph. Eur., the microbial action limit is 10 CFU/100 mL, and the level of bacterial endotoxin must be <0.25 EU/mL. In order to show that the distillation unit is functioning as designed, it is good practice to monitor the endotoxin levels of the feed water to ensure that the challenge does not exceed

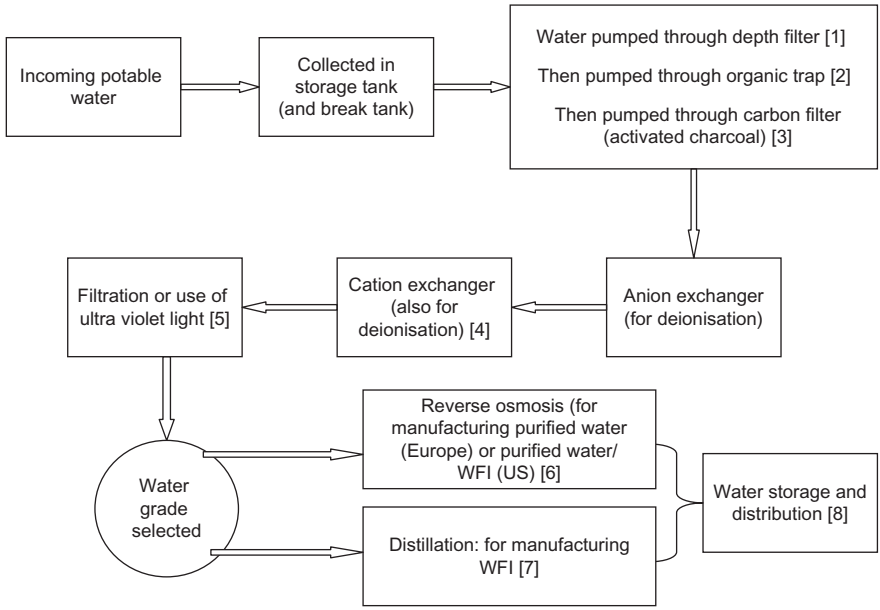

Figure 10.1 Diagrammatic representation of a pharmaceutical water system.

250 EU/mL (in order to generate WFI with an endotoxin level of less than 0.25 EU/mL, that is a three-log reduction has been achieved).

Figure 10.1 shows a typical schematic for the generation of pharmaceutical grade water.

In relation to Figure 10.1:

1. The depth filter is made from granular anthracite, washed sand, and gravel. The filter requires regular regeneration by backwashing;
2. The organic trap is resin, to which organic matter is removed from the water by binding;
3. The carbon filter absorbs residual organic materials, such as chlorine;
4. Anions, for example, Cl^- and SO_3^{2-}, are exchanged with hydroxyl (OH^-) counter ions from the anion exchanger;
5. Cations in water, such as Na^+, Mg^{2+}, and Ca^{2+}, are retained by displacing H^+ ions from the exchanger. Both the cation and anion exchangers are monitored by measuring restivity. As ions are exchanged and the process progresses, restivity should increase;
6. Filtration is performed using a 0.22 µm filter; ultraviolet light typically is of a wavelength of 254 nm;
7. Reverse osmosis uses a semipermeable membrane (made from polymers such as cellulose acetate or polyamides), which is permeable to water but not to microbial contaminants. Osmosis allows the movement of water across the membrane, moving from a solution of lower solute concentration to one of higher solute concentration, through the force of osmotic pressure. In some processes, double reverse osmosis is performed for additional assurance that microbial contaminants have been removed.
8. With distillation water is heated so that it condenses and is converted back into water; this process removes most impurities along with bacterial endotoxin.

9. The pharmaceutical grade water is held in a sealed storage vessel and fed around a network of pipes (distribution loop) to provide the manufacturing area. Unused water is returned to the storage vessel. The quality of the water is protected by a fast flow rate (typically greater than 9 ft/s; 2.7 m/s), which creates a constant, turbulent flow to minimize microbial attachment, and biofilm formation. Pipes are also designed to avoid dead-legs (to avoid stagnant water, as discussed below). To maintain the quality of the pipework, regular passivation and de-rouging are required. Furthermore, valves, especially those associated with user points, should be cleanable, free-draining, and not prone to leakage [5].

10.3 The microbial ecology of water

All water presents some form of microbial risk in that water provides a means for microorganisms to reproduce, and it is also an effective way of transferring microorganisms across distances. With the types of water found in the pharmaceutical facility, there are variations to the microbial ecology. With in-coming potable water, the microbial composition will vary depending on two factors. The first factor is the catchment area. Water used for the production of potable water is collected from vastly differing environments. These range from the nutrient-poor (oligotrophic) upland rivers where the microbial count, even using direct counting methods, will seldom exceed a few thousand CFU per milliliters, to the nutrient-rich (eutrophic) regions of lowland rivers, where counts can exceed 1 million per milliliters. The second factor is the season; the levels of microorganisms in natural waters follow a seasonal distribution curve controlled by the amount of available nutrients and temperature [6].

The composition of the microbial flora in the source water will be predominantly Gram-negative, containing prosthecate bacteria (bacteria that possess appendages), such as *Hyphomicrobium*, *Caulobacter*, *Gallionella*, and *Pseudomonas* species [7]. Bacteria are not the only microorganisms that inhabit source waters; the ecosystem will include fungi, protozoans, and algae. Contamination from land run-off and sewage contamination may add any number of potential pathogens [8].

With pharmaceutical water, through a process of increased purification, the complexity of the microbial ecosystem decreases as the diversity of the microorganisms prevalent within the water system correspondingly decreases. This is due to a reduction in niches within the ecosystem [9]. With purified water and WFI, the numbers of microorganisms should be very low. Generally, it is unlikely that the water generation will result in high levels of microorganisms—if the system is functioning well. Contamination tends to be introduced through poorly designed pipes or at user points.

10.4 Design and control of water systems

As indicated above, most microbial problems arise from the storage and distribution of the water rather than its generation. Primarily this arises through the development of biofilms, and, once established, biofilms can be extremely difficult to remove. In addition to distribution, improperly maintained generation components, such as carbon

beds, softeners, reverse osmosis membranes (components illustrated in Figure 10.1), can also contribute to subsequent contamination downstream within the distribution system. Therefore, the design of water systems is of great importance.

An example of the importance of good design practices can be illustrated with WFI systems. WFI systems often stretch for hundreds of meters, sometimes across multiple floors; and the systems require high water flow rates [10]. Appropriate placement of outlets should be built into the design to assist operational logistics, to ensure representative sampling and the minimization of dead legs. As a part of biofilm control (as discussed below), stainless steel pipework or good quality plastic piping (such as polyvinylidene fluoride) will be used. In addition, sanitary design for valves is important. The design must minimize opportunities for water flow stagnation, for example, dead legs, or sites that allow for residue accumulation [11].

The requirements for well-designed water distribution pipes include:

(a) Smooth internal surfaces in tanks and in pipe-work. Microorganisms adhere less well to smooth surfaces than to rough surfaces. Pipe joints and welds can disrupt smoothness;

(b) Continuous movement of the water in tanks and rapid flow in pipework. Where shear forces are involved, microorganisms adhere poorly to surfaces. Where there is no movement of the water, there is no shear, shear increases with speed of flow;

(c) Avoidance of areas where water can remain stagnant.

i. These include "dead legs"—water may stagnate in branch pipes branch from a circulating main if the length of the branch is too long to allow the turbulence of the flowing main to disturb the contents of the branch pipe. Here the principle is to always minimize the length of branch pipes;

ii. Water can also remain stagnant in valves, particularly at user points and especially at user points which are not in frequent use. This problem can be counteracted by the use of so-called hygienic or "zero dead leg" valves. While these are significantly better than the alternatives (say ball valves), they should not lead to a false sense of security for they can harbor endotoxin-shedding biofilms;

iii. Ring mains should be sloped (have "drop") from point of origin to the point of return to ensure that systems are completely drainable;

(d) Avoidance of leakage. Water leaks can cause bridging of water to the external environment through which bacteria may enter the system. Storage tanks should be equipped with filter on their air vents to prevent air-borne microbiological ingress. They may even be held under a "blanket" of an inert gas such as nitrogen;

(e) Controlled temperature storage and distribution is required for WFI systems. The risks of endotoxin-shedding biofilms despite the best attempts at control above are thought to be so consequential that the regulatory bodies require the temperature of storage and distribution to be maintained higher than 75 °C. It should, however, be considered that 75 °C is too high a temperature for most pharmaceutical formulation purposes. This means that user points are generally equipped with some form of cooling mechanism. It should be noted that heat exchangers used for this purpose may be a source of endotoxin and bacterial contamination and may thus cancel out many of the benefits of high temperature circulation.

In contrast to WFI, purified water systems can be maintained as a hot system or a cold system. Unlike WFI systems, purified water systems are typically cold systems and rely upon ultra-violet light and in-line filters to maintain microbial quality. Ultraviolet radiation (254 nm) is used for the disinfection of water of good optical clarity, and works particularly well in a re-circulating system where water flows over

a multiple lamp system. If contamination occurs, both WFI and purified water systems should have the capability to be sanitized either by steam or by chemicals such as chlorine dioxide [12].

Even a good design can go wrong; therefore, operational control is also important. Operating procedures should require outlets to be flushed before usage to ensure use of the circulating water and to remove possible stagnant water or contamination from the surface of the outlet. Importantly, in the context of microbiological sampling, the flushing of outlets prior to sampling for monitoring purposes should be equivalent to that applied in operational use.

The use of hoses and temporary piping is a major source of contamination to product in during manufacturing, and therefore, their use should be minimized. Where used they should be subject to appropriate controls to minimize the risk of contamination from this source. For example, they should not be left on the outlets; they should be dried after use, hung vertically in appropriate locations to ensure free drainage, monitored, and, on a daily basis, cleaned, sanitized, and replaced.

With both the design and operation, the components of the storage and distribution system should enable validated cleaning and sanitization to be conducted.

10.5 Qualifying water systems

When a new water system is designed, it should be subject to formal qualification. Water systems require qualification based on bioburden, bacterial endotoxin, and organic and inorganic impurities [13]. Qualification steps include an operational qualification, where each outlet is monitored for a minimum of 2 weeks across each working day prior to the water being released for production; and a two-phase performance qualification. Phase I of the qualification will consist of 4 weeks of sampling as the water is being used by production, where samples should be taken at different times of production operations. Phase II is an assessment of the water over the course of 1 year. During the qualification, all out-of-limits results must be investigated and a root cause established.

10.6 Microbial contamination

Pharmaceutical water systems can become contaminated. Contamination can relate to special cause events, such as contamination at a user outlet (e.g., a hose left in a sink); and to common causes, which are systematic issues affecting the entire water system. A typical common cause can be caused by the formation of a biofilm.

10.6.1 Biofilms

Biofilms are made from a complex consortia of microorganisms organized within extensive exopolymer glycolices. Once formed, biofilms can be very difficult to remove, requiring a combination of heat and chemical treatments. The problems caused

by biofilms include biofouling, biodeterioration, and physical blockage of industrial pipework and heat exchangers in water systems [14]. Microbial biofilms develop when microorganisms adhere to a surface by producing extracellular polymers that facilitate adhesion and provide a structural matrix. While the majority of bacteria are trapped within a biofilm, the biofilm will constantly generate bacteria that are released as free-floating individual cells and parts of the biofilm may slough off in clumps. As water is used and flows through the pipework or tap containing the biofilm, then the contamination risk arises at the point at which the water is used.

The steps involved in biofilm formation are:

1. individual cells populate the surface (initial attachment);
2. extrapolymeric substances (EPS) are produced, and attachment becomes irreversible;
3. biofilm architecture develops and matures;
4. single cells (or clumps of cells) are released from the biofilm.

The primary concern is that biofilms are highly recalcitrant and extremely difficult to remove once established.

Various design features may be utilized to prevent the development of biofilms and control contamination. The design of the system should include continual circulation with adequate flow rate to aid the prevention of biofilm formation (typically 1–3 m/s). Other common design features are the capacity to heat the water to elevated temperatures (as discussed above, elevating WFI to 75 °C or hotter) for sanitization purposes and the inclusion of high intensity ultraviolet (UV) light lamps. The inclusion of UV lamps downstream of potential microbial reservoirs, for example, carbon beds and softeners, has the added advantage of enabling ozone to be used for sanitization purposes. Where UV lamps are used, they should be regularly checked and maintained to ensure they are clean and provide the correct wavelength and energy output. The inclusion of filters within the distribution loop is difficult to justify and is not advisable.

Older systems, without such design features may require regular disinfection using an oxidizing agent to control biofilm (e.g., ozone, hydrogen peroxide, or hypochlorite). Each of these methods has disadvantages, including the need for additional design considerations (such as UV light to destroy ozone or extensive flushing is required to remove chemical residues if hypochlorite is used, which is expensive and disruptive [15]).

10.7 Microbiological sampling and testing

To assess the effectiveness of a water system, microbiological sampling is necessary. This includes taking samples of the incoming water at point of entry, the generation process used to produce water, the distribution tanks, and user outlets for both purified water and WFI. The frequency of sampling should be sufficiently high as to allow for meaningful trend analysis to be conducted. For this, at least some level of daily sampling is required (although each user point does not need to be sampled each day).

It is important that samples for microbiological analysis are taken appropriately. Good sampling practice includes taking samples through freshly autoclaved tubing, allowing

outlets to be flushed for a standardized time or volume, and by taking the sample via good aseptic technique. Another aspect that is important is either testing samples within 2 h of the sample having been taken, or holding the samples at 2–8 °C prior to testing. The hold period, which would not ordinarily exceed 24 h, should be validated [16].

In terms of methodology, membrane filtration is the method of choice since a large volume of the sample is assessed. For potable water and water that is being processed through the generation plant (such as deionized water), some users elect to perform 1-mL plate counts. With microbiological agars, a standard plate count agar (PCA) is commonly used to assess potable water [17]. With purified water and WFI, the European Pharmacopoeia recommends Reasoner's 2A medium (R2A) agar. With the USP, no medium is recommended and the selection of the most appropriate culture medium rests with the site microbiologist (and ideally through a validation study). Nonetheless, the use of R2A is commonplace.

The widespread use of R2A is because it has long been recognized that total aerobic counts performed on water samples using low nutrient media (and preferably low 20–25 °C incubation temperatures) give much higher results in terms of microbial counts [18]. About 5–10-fold increases are often the norm when R2A is compared with general nutrient agar, such as tryptone soya agar [19]. The reason for this difference in magnitude is because bacteria undergo physical alterations and a metabolic downshift to survive in oligotrophic environments. Multiple genes are involved in this metabolic switching. This leads to bacteria in water being found in one of the two physiological conditions, and these conditions affect the ability of the bacteria to be recovered on different nutrient media.

Free-swimming bacteria (planktonic phase) are common to water systems, and it is these microorganisms that are collected and counted in water monitoring programs. The planktonic phase is energy expensive; it is a distribution strategy, but it is not a good survival strategy for starvation conditions. Survival mode is a switch from the planktonic phase to the benthic phase. Bacteria in the benthic phase lose motility, attach firmly to surfaces and start producing extracellular polymeric substances (EPS), which is the basis of a biofilm (as discussed earlier). EPS concentrate trace growth factors and afford protection from antagonistic agents such as biocides and heat treatments. Frequently reductions in cell size occur. Microorganisms in the benthic phase are often very difficult to culture on complex, rich media and they have also been described as viable but non-cultivable. Such bacteria can, however, be cultivated on low nutrient medium at lower incubation temperatures and extended (10–14 days) incubation times. Nonetheless, the value of a total aerobic count result obtained 14 days later is certainly questionable. Therefore, the Ph. Eur. "compromises" with the requirement for an incubation temperature of 30–35 °C and an incubation time of 5 days [20].

Even at 5 days, the value of an enumeration result is questionable, particularly so for water, which provides a dynamic environment. If the count exceeded an action level, it did so 5 days previously and possibly remained out of specification for the next 4 days. The net result is a difficult evaluation of the microbial quality of products manufactured during that period. This is why the emphasis should be on trend analysis rather than on individual results. It is also on this basis that there has been considerable investment and development in rapid and alternative microbiological methods (see below).

From this discussion about agars, it is important to appreciate that whatever cultural technique is used, it will only show a fraction of the microbial population in the sample. For that reason, the specifications for water counts are described as action limits; they are not considered to be pass/fail limits. If an action limit is exceeded, its impact on the product must be evaluated, but this does not often lead to batch rejection.

For WFI, water systems need to be assessed for bacterial endotoxins. This is undertaken through *Limulus* amebocyte lysate (LAL) methodology, with a limit of 0.25 EU/mL applied. LAL is discussed separately in this book. Although not directly related to microbiology, water systems are also examined for their chemical purity. Arguably, the most important examination is with total organic carbon (TOC) [21]. TOC is the amount of carbon bound in an organic compound and is often used as a nonspecific indicator of water quality or cleanliness of pharmaceutical manufacturing equipment. Although there is not a direct relationship with microorganisms, high levels of TOC may infer bacterial growth within the water system.

10.8 Action and alert limits

For the monitoring of water systems, appropriate alert and action levels should be set for both bioburden assessment and for levels of bacterial endotoxins. Action levels, where appropriate, are typically drawn from the pharmacopoeia or national water standards, whereas alert levels are assessed by pharmaceutical organizations, based on a review of historical data. The European and World Health Organization pharmacopoeial monographs for each type of water include statements on action limits; whereas the equivalent chapters within the USP recommend that "appropriate" monitoring limits be set. To set levels using historical data, ideally 1 year of data (or more) is analyzed in order to account for seasonal variations. There are different ways to calculate alert levels; one example is to take the 95th percentile.

Alert and action levels can be defined as follows:

- Alert limits are levels that when exceeded indicate that a process may have drifted from its normal operating condition. Alert limits constitute a warning and do not necessarily require a corrective action.
- Action limits are levels that when exceeded indicate that a process has drifted from its normal operating range. Exceeding an action limit indicates that corrective action should be taken to bring the process back into its normal operating range. Alert limits should be set below action limits.

If an action limit has been exceeded, the impact on the product(s) involved needs to be carefully investigated and evaluated. Furthermore, action and alert levels are useful markers for trend analysis. To assess the microbiology of water systems in a meaningful way, the collected data should be examined for trends; ideally on a monthly, quarterly, and annual basis (the latter allows an assessment of seasonality). Care must be taken when assessing microbial counts using traditional graphical

tools, since such control charts are established on the basis that the data plotted are normally distributed. Microorganisms in water tend to follow Poisson distribution. Therefore, microbial counts require transforming prior to the results being plotted onto trend charts (such as by taking the square root or calculating the logarithm to base 10) [22].

When upward trends or action level excursions are recorded, investigations should be undertaken. The investigation should establish the cause of the excursion, and if possible, eliminate it. The evaluation should examine the impact on the product and its ability to withstand microbial challenge, as well as the patient group and their susceptibility to infection (this assessment requires identification of the contaminating microorganism). With action limit excursions, the investigation and evaluation should be carefully documented, and a justification for product release or rejection should be prepared. Some areas for investigation are displayed in Table 10.1.

Table 10.1 Table showing potential areas for the examination of water system problems in the event of microbial excursions

Main area	Areas for investigation
Sampling	Aseptic technique
	Adventitious contamination (type of microorganisms)
	Consumable/reagents/media—satisfactory?
	Were any pipes/valves leaking at time of sampling?
	Condition of sampling outlet
	Burst or leaking pipes
	Loss of pressure
	Identify who took the sample
	Staff training
	Integrity of container
	Interventions
	Transportation
	Storage
	Design of sample valve
	Check flushing
	Storage
Test method	Consumables—integrity/expiry date
	Reagents/media—storage/expiry date?
	Equipment—service/calibration
	Aseptic technique
	Test method—procedure followed?
	Incubation conditions
	Test controls
	Interpretation of result/calculations
	UDAF calibration/settle plates/airflow?
	Tubing—present/absent, does the tubing appear worn?

Continued

Table 10.1 Continued

Main area	Areas for investigation
Housekeeping/ specific outlet issues	Integrity of point: e.g., leaks, state of values, joints on tubing-outlet connections Past history of point reviewed Usage of point Temperature of outlet and/or sample Tubing—storage Steaming/sanitization satisfactory? Any issues for water supply?
Use	Establish what the water has been used for (e.g., direct product contact, such a dilution of product or buffer preparation?)/review usage of the point Any problems with plant samples?
Plant maintenance	Steaming/sanitization performed to set frequency? Review of plant history log Check flow rates
Design	Check loop temperature Check for dead legs Check valve design and maintenance Check task turnover rate Check task levels Check filters and change dates

10.9 Undesirable (objectionable) microorganisms

In addition to the examination of microbial counts, some facilities examine water systems of the presence of so-termed "objectionable microorganism." An "objectionable" microorganism is any microorganism that can cause infections when the drug product is used as directed or any microorganisms capable of growth in the drug product. In most situations, this can be translated to the absence of *P. aeruginosa* and *Burkholderia cepacia*, and the absence of any *Pseudomonas* spp. in nonsterile ophthalmic preparations. Occasionally, screening for *E. coli* is added as an indicator of fecal contamination (although it is unlikely that in-coming water is contaminated with such organisms). However, each pharmaceutical manufacturer must determine which microorganisms are classed as "objectionable" in relation to a specific process. The rationale of judgement will have to be based on product application and patient group vulnerability.

Examination for specified microorganisms requires the use of selective media and/or enrichment steps; or, alternatively, specialized test kits. The recovery of suspect microorganisms from general test agar and identifying them is not acceptable. This is because the sensitivity of detection will be too low.

10.10 Rapid microbiological methods

Rapid (or alternative) microbiological methods have made some progress with water testing. The reason why such methods attract attention is not only a shorter time-to-result, but also because they detect a greater proportion of the microorganisms that are potentially present. If samples of water were tested by heterotrophic plate count and by direct counting methods (such as flow cytometry) the results in CFU/milliliter for the plate count would, in all probability, be in the range of 0.1–10% of the direct counts. This is because many of the microorganisms in water systems are unable to grow on plate count media; for some microorganisms, the media is too rich, and for other microorganisms, the cultural conditions are unsatisfactory. Improved recoveries are seen on R2A for prolonged periods at lower temperatures (as discussed above); however, the phenomenon of "viable but nonculturable microorganisms" means that many microorganisms found in water will not grow using cultural methods.

A range of rapid methods is available for the screening of water samples for indicators of contamination based on chromogenic, fluorogenic, or chemiluminogenic substrates. For example, with the examination of coliforms, the assays are based on the assumption that β-D-galactosidase and β-D-gluconidase are markers for coliforms and *E. coli*, respectively [23]. An alternative approach is with light scattering methods that can be used for the detection of water pathogens. With this method, as the slipstream passes through the flow cell, it also passes through a laser beam.

10.11 Microbiological assessment

The results from microbiological monitoring will typically be satisfactory over the course of 1 year. Sometimes over action results will be recorded from user outlets; more often these incidents are the result of poorly maintained sinks (where there is a risk of splash-back) or through the local management of hoses. Such "special causes" events are rarely of a concern unless they occur in succession, as shown through a repeat sampling regime.

When systematic problems occur (including common causes such as biofilm formation), it is of great importance that microbiologists understand the basis of water system design. This is a key to root cause investigation. Concerns can also arise with the generation plant. With the production of pharmaceutical grade water, one of the weaknesses is that the resin beds can actually add microorganisms to the water if they are not properly maintained. A second concern is with the ion exchange process; here there is a risk if the ion exchange process does not remove microorganisms.

In order to gain sufficient oversight, the microbiologists should regularly review data from the water system and examine the data for trends. An example of a trend chart showing adverse drift is shown in Figure 10.2.

In Figure 10.2, the chart indicates the start of an adverse trend (emphasized by the addition of a linear plot trend line), thus an out-of-control situation, with a series of points rising above the upper control level. With the chart, the mean count has been

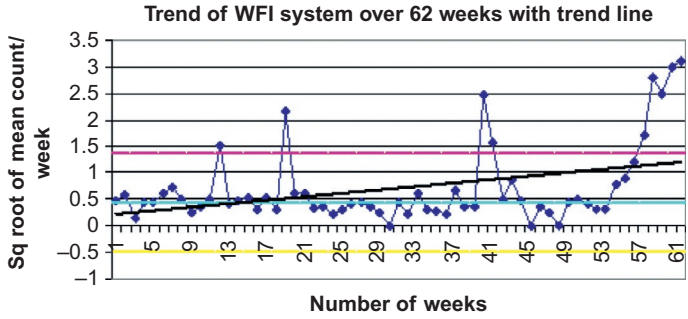

Figure 10.2 Plot of a microbial water system.

transformed through taking the square root of the mean count for each week. This is in order to approximate normal distribution.

In the example chart, the organization should have taken action concerning the water system and have closed the system down for investigation and formulation of appropriate preventative action. It is for this reason that adverse drift should always be investigated.

10.12 Summary

This chapter has examined pharmaceutical water systems. The chapter, in keeping with the theme of the book, has focused on the microbiological aspects of water systems. This concern with microbiological contamination has prevailed through considerations of system design, the risks presented from biofilms and with microbiological sampling. In examining these areas, it is clear that the site microbiologist should play an active role with the control and management of the water system. Much of this is based on a thorough examination of the data and in understanding how the production cycle impacts upon this, including high and low usage and seasonal variations. Water is of critical microbiological concern and it is important to be vigilant.

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