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Questions and answers on production of water for injections by non-distillation methods – reverse osmosis and biofilms and control strategies

Draft

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This set of questions and answers is intended to provide preliminary guidance until such time the on-going revision of Annex I of the GMP guide is complete.

Comments should be provided using this template. The completed comments form should be sent to adm-gmdp@ema.europa.eu

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Introduction

Following discussions over the last 2-3 years around the revision of the European Pharmacopoeia (Ph. Eur.) Water for Injections (WFI) monograph (0169), the Water Working Party concluded that there was evidence to support a revision of the monograph, which proposes to take account of current manufacturing practices using methods other than distillation for producing water of injectable quality.

The Ph.Eur. monograph (Monograph 169) was revised to include, in addition to distillation, reverse osmosis (RO) coupled with suitable techniques, for the production of WFI.

WFI monograph 169 states:

> Production
>
> Water for injections in bulk is obtained from water that complies with the regulations on water intended for human consumption laid down by the competent authority or from purified water. It is produced either:
>
> - by distillation in an apparatus of which the parts in contact with the water are of neutral glass, quartz or a suitable metal and which is fitted with an effective device to prevent the entrainment of droplets; or
> - by a purification process that is equivalent to distillation. Reverse osmosis, which may be single-pass or double-pass, coupled with other appropriate techniques such as electro-deionisation, ultrafiltration or nanofiltration, is suitable. Notice is given to the supervisory authority of the manufacturer before implementation.
>
> For all methods of production, correct operation monitoring and maintenance of the system are essential. In order to ensure the appropriate quality of the water, validated procedures, in-process monitoring of the electrical conductivity, and regular monitoring of total organic carbon and microbial contamination are applied.
>
> The first portion of water obtained when the system begins to function is discarded.
>
> Water for injections in bulk is stored and distributed in conditions designed to prevent growth of micro-organisms and to avoid any other contamination.

The purpose of these Questions and Answers is to provide clarification and guidance in relation to the use of reverse osmosis in the manufacture of Water for Injection (Part I) and also to provide more detailed guidance on the control of Biofilms (Part II).

Part I Production of wfi by non-distillation methods – reverse osmosis

1. The monograph requires that notice is given to the supervisory authority of the manufacturer before implementation. Who is the supervisory authority?

For a manufacturing site located in the European Union the supervisory authority is the relevant competent authority responsible for GMP oversight in the Member State concerned.

For a manufacturing site located in a third country engaged in the manufacture of medicinal products (produced using WFI) which are exported to the European Union, it is the relevant competent authority responsible for GMP oversight in the Member State of the importer in the European Union. If affected products are exported directly to more than one Member State of the European Union, any one of the

respective supervisory authorities should be notified. Notification to EU authorities is without prejudice to any similar obligation the manufacturer might have towards the relevant authorities of the country in which it is located.

By analogy for the sole purpose of this guidance, a manufacturing site located in a third country engaged in the manufacture of medicinal products (produced using WFI) which are exported to the European Union and where a Mutual Recognition Agreement, or equivalent agreement exists between the country concerned and the European Union and the affected products are within the operational scope of the agreement, it is the relevant competent authority responsible for GMP oversight in the country concerned.

2. What are the main concerns around the use of reverse osmosis to manufacture WFI?

The main concerns around the use of non-distillation methods – Reverse Osmosis, for the manufacture of WFI relate to the microbiological quality of the water produced as well as the control mechanisms in place to minimise the risks associated with microbiological proliferation and/ or by-products throughout such a system which is not easily detected.

RO systems typically operate at ambient temperatures and as such offer an ideal environment for the formation of a biofilm. Biofilms are notoriously difficult to remove, because they protect flora contained within against the action of shear forces and disinfection chemicals. In addition, incompletely removed biofilms lead to a rapid regrowth and proliferation as well as increasing the likelihood of microbiological by-products throughout a system.

3. What are the main elements that should be considered in the design of such a system?

The system design should be in such a manner as to minimise the risk of microbiological contamination and proliferation.

Control Strategy:

A robust control strategy should be developed in parallel with the design considerations. The control strategy should take account of the risks involved in the use of RO to manufacture WFI, the measures to be taken to address those risks and additionally the various control measures required to be implemented in order to provide adequate assurance of the water quality, or that the specific control measures in place are designed in order to enable identification of any issues which may impact the quality of the water produced.

Additionally, the potential for biofilm formation should be appropriately assessed and measures put in place to minimise the formation of biofilms within a system. See section 2 – Biofilm control strategy.

Materials of construct:

The materials of construct for the generation and distribution systems must not be reactive, additive or absorptive to such an extent that it will affect the quality of water produced.

The distribution and storage systems should be designed as to permit routine steam sanitisation along with routine chemical sanitisation and in accordance with other good design practice to minimise areas of reduced flow.

Pre-treatment:

Microorganisms entering an RO system encounter a large membrane surface where the dissolved organic nutrients of the water are concentrated. Therefore the quality of water entering the system is critical. Appropriate pre-treatment is necessary to:

- Ensure adequate removal of organic particles and microbiological impurities – Use of ozone should be considered as it is a powerful antioxidant that controls microbial growth and reduces the concentration of organics due to oxidation. Its use requires compatible materials of construction for the water system.
- Control of scaling - usually controlled by use of ion exchange upstream of the membrane
- Control of fouling – use of depth or media filtration is typically employed and is often the first step in a pre-treatment system
- Removal of microbial control agents – Chlorine can cause degradation of the membranes and its removal is necessary – typically removed during the latter stages of pre-treatment as its antimicrobial properties aid with minimising microbial proliferation throughout the pre-treatment stages
- Residual free chlorine can be reduced by activated carbon or chemical reducing agents such as sodium metabisulfite (SMBS) commonly used for removal of free chlorine and as a biostatic. Residual free chlorine can be detected with oxidant-reduction potential electrodes (ORP). Other oxidizing agents such as chlorine dioxide, hydrogen peroxide, ozone, and permanganate are capable of damaging RO membranes also if not used properly.

Pre-treatment of water is essential in order to minimise the impact to the RO membranes. Techniques such as deionisation, water softening, descaling, pre filtration, degasification (can be located between the stages of a double pass RO system), nanofiltation, electro-deionisation, ozonation, UV treatment and micro-filtration should all be considered during the design phase to assure the quality of the water produced.

Pre-treatment is necessary to ensure that the feed water will be of an adequate quality to feed to a final treatment step, thereby protecting the membrane, minimising membrane degradation and aid with minimising the risks associated with microbiological proliferation and biofilm formation.

The quality of RO feed water should be monitored.

RO Membranes:

RO membranes should be robust enough to permit routine high temperature sanitisation along with routine chemical sanitisation. *RO membrane development must also be taken into consideration and where such evolution of membrane resistance permits, higher temperatures (>120°C), pressures and a more harsh chemical sanitisation regime must be applied. Systems should be designed to allow for such changes to be implemented.*

Systems should be in place to test membranes routinely for any potential integrity breaches that could lead to a significant contamination event.

Use of Double pass RO membranes should be considered as an added assurance of the maintenance of the quality of the water produced.

Additional techniques to be considered

Coupled with these further techniques post RO membrane should be considered such as nanofiltration, electro-deionisation and ultra-filtration (known to have an endotoxin reducing capability).

Microfiltration(MF)/ultrafiltration(UF) offers advantages in that it can remove microorganisms, which are sometimes very difficult to remove by standard techniques. The MF/UF membranes should be made from a chlorine-resistant material to withstand periodic sanitisation.

Total Organic Carbon (TOC)

On-line TOC meters must be employed as a prerequisite to the control strategy and located at various positions within the RO water system. The location of on-line TOC should be based on risk assessment. Locations to consider:

- Feed water monitoring – assess for seasonal or unanticipated quality changes that could negatively impact the pre-treatment system capabilities or cause a significant increase in membrane fouling
- Monitoring downstream of pre-treatment – can aid with verification of satisfactory equipment operation and aid as an advanced warning of degradation of the pre-treatment systems
- Monitoring post RO membrane and UV lights – can aid with detection of compromised membranes or the need for UV lamp replacement
- Monitoring post final treatment step to verify acceptable water quality prior to delivery to the storage tank. TOC meters are often located on the return loop of the distribution system, prior to recirculation back to the storage tank.

System design should be such that there is an automated diversion through a recirculation system back through the pre-treatment process and final purification equipment when the quality of the water produced is outside the acceptable limits this should also result in reporting under the Pharmaceutical Quality System so that the frequency of such excursions can be monitored and also the root cause investigated appropriately.

When on-line TOC systems fail, robust corrective measures should be put in place that will assure the ongoing quality of the water produced.

Appropriate alert limits should be established based on the data generated during the system performance throughout the qualification phases and commensurate with operating capabilities of the system. Alerts should be reassessed routinely to enable, where possible, a tightening of those control limits. Increasing of such limits is not good practice and may mask a failing system.

Conductivity

On-line conductivity meters must be utilised as a prerequisite to the control strategy and be installed at various locations within the RO system. The location of these meters should take account of the locations specified above under TOC but should also consider the monitoring of RO concentrate and permeate in order to aid with determination and trending of percentage rejection from the system in operation. Changes in rejection percentages can be an indication of membrane failure, seal failure, improper pH, feed pressure issues and increased scaling or fouling.

Trend data should be reviewed routinely in order to determine the potential for deterioration in the system.

When on-line conductivity systems fail, robust corrective measures should be put in place that will assure the ongoing quality of the water produced.

Sanitisation.

The system should be designed to allow for routine sanitisation. The frequency should be determined based on risk assessment and on the data gathered during the qualification of the system.

Monitoring of the flora in the system should be considered to allow adaptation of the sanitisation procedure, based on the resistance of the concerned microorganisms.

The system should be pressure rated to enable routine steam sanitisation throughout the distribution loop and storage tanks. The RO membranes are currently not designed to withstand pressurised steam, but those that are capable of withstanding high temperatures are available and should be utilised in order to allow for routine high temperature flush through of the system in conjunction with routine chemical sanitisation.

Use of the following chemical sanitising agents should be considered as part of the control strategy:

- Peracetic acid
- Sodium Hypochlorite
- Hydrogen Peroxide

Appropriate contact times need to be established.

Use of Ozonation should also be incorporated into the design of such a system. Ozone is an even stronger oxidizing agent than chlorine and it decomposes readily. The resistance of the materials of construction against ozone must be considered. Usually, stainless steel is employed; it is unlikely that a distribution system with non-stainless steel components would be acceptable. Ozone can eliminate a wide variety of inorganic and organic materials and aid with maintaining an appropriate level of microbiological control.

De-ozonation must be performed carefully to protect the membranes. Ultraviolet irradiation is typically utilised for this purpose.

4. What approach should be considered for the qualification of such a system?

The approach to system commissioning and qualification should follow good engineering practice. The approach should be developed to provide the necessary evidence that the design of the water system is in line with that intended in order to assure the quality of the water produced during routine operation.

Performance of the system must be proven over an extended period of time and the sampling programme employed must be sufficiently robust to take account of this.

The initial validation period of the water system where testing is carried out on all points should be extended to build confidence that the system is operating as designed.

Similarly, subsequent phases of system validation should be robust and capture significant data to verify ongoing capability of the system.

5. What type of sampling regime should be employed during qualification and during operation?

The sampling regime during the initial stages of qualification should take account of the critical points within the system. Such locations to consider include:

- Feed / raw water source
- Stages of pre-treatment
- Pre and post RO membrane
- Post final purification phase
- Storage tank
- All user points
- Return loop post final user point

Typically during initial phase, qualification testing of all of the above points should be sampled and tested daily for a specified period of time in order to assure the correct installation and operation of the system.

The next phase of sampling should also take account the above locations. The sampling frequency should be designed in a manner to assure satisfactory performance of the system over an extended period of time. Typically this is conducted over a year to take account of, for example, seasonal variations associated with feed water supply.

During routine operation the sampling regime (frequency and locations) should be designed in a manner to assure satisfactory continued performance of the system and ultimately assure the quality of the water produced.

Daily sampling of the system should be employed for all user points utilised on the day, the return loop as well as consideration of inclusion of points both pre and post the RO membranes.

Volumes sampled for microbiological monitoring should be justified and commensurate to test requirements.

6. What testing should be employed during initial qualification and routine operation sampling?

Testing should be conducted in line with Ph.Eur. Monograph 169 '*Water for Injections*'

Use of rapid microbiological methods should be employed as a prerequisite to the control strategy to aid with rapid responses to deterioration of the system.

Article 23 of Directive 2001/83/EC states:

- "…the authorisation holder must, in respect of the methods of manufacture and control…take account of scientific and technical progress…"

Methods to be considered should include:

- Rapid Endotoxin testing – use of more sensitive and point of use test methods
- Quantitative microbiological test methods – in line with Ph.Eur. 5.1.6 monograph 'Alternative Methods for control of Microbiological Quality'.

Due consideration should be given to employing alternate methods for the rapid quantitative determination of the contamination levels existing within the water system. The validation of such system should be in line with the above referenced monograph.

Use of alternative/ rapid microbiological test methods should be employed as part of the overall control strategy for the system.

Appropriate alert limits should be established based on statistical analysis of data. Trend data should be reviewed routinely and any adverse trend should be appropriately investigated. The review of trend data should not only take account the % alert and % actions occurring but also review of the quantitative and qualitative (identifications) raw data.

Alerts should be reassessed routinely to enable, where possible, a tightening of those control limits. Increasing of such limits is not good practice and may mask a failing system.

7. What are the expectations for preventative maintenance on RO systems used for the production of WFI?

A robust system for preventative maintenance of such systems should be designed as part of a control strategy in order to minimise the risks associated with microbiological and / or by-product proliferation.

The planned maintenance system should incorporate routine regeneration of pre-treatment systems, replenishment of resin beds (as required), change out of filters, gaskets, seals and RO membranes at a defined frequency or following adverse indicators as well as routine sanitisation of such systems. Detailed inspection checks should be incorporated into the routine planned maintenance to take account of the potential for the formation of biofilm within the system:

e.g. Inspection for leaks within the system, inspection of the condition of gaskets and seals.

Performance of the RO membrane(s) should also be assessed as part of the routine planned maintenance approach including determination that the pressures and flow rates are in line with the satisfactory operation of the system in order to maintain the quality of water produced to the appropriate standard.

Part II Biofilms and control strategies

1. What is a biofilm?

Biofilms occur in both natural and industrial settings. They are ubiquitous.

They can typically be found in air compressor and supply systems, water systems, heat exchangers, RO membranes, ion-exchange resins, piping, O-rings, gaskets and more or less anywhere that an aqueous or moist environment exists.

Sites for biofilm formation include all kinds of surfaces: natural materials above and below ground, metals, plastics, medical implant materials—even plant and body tissue. Wherever you find a combination of moisture, nutrients and a surface, you are likely to find biofilm.

They are typically a mass or group of varying species of micro-organisms. They are formed when these organisms adhere to the surface in a moist environment. These in turn secrete extracellular polymeric substances (EPS) that act as an anchor to the surface as well as to other micro-organisms of various species. This in turn allows them to develop complex three-dimensional structures or communities. Biofilms typically follow similar routes for formation and spread:

- Attachment
- Colonisation

- Growth
- Detachment

The development of biofilms on otherwise clean surfaces (i.e., surfaces that are free of organic and inorganic contaminants) proceeds through a 4-step process:

1. Sorption of trace organic and inorganic compounds to form a conditioning film, which may serve as an organism recognition factor in the initial phases of attachment.
2. A reversible primary attachment, mediated by advective transport processes and/or chemotaxis, which is the movement of an organism in response to a chemical gradient.
3. Surface-division also referred to as colonisation.
4. Synthesis of EPS, which stabilises the sessile population.

Such biofilm communities can communicate via quorum sensing and in the presence of certain danger or death, induce secretion of protective metabolites within the structure of the biofilm signalling and inducing a form of protection to the layers within the biofilm layer.

Little is understood of the extracellular polymeric substances and metabolites produced by these organisms. Also, little is understood of the cellular debris which remains after cell death. There are no specific Ph.Eur tests specified to test for some of these EPS and metabolites. Some of these include Exotoxins and Bacteriocins (piocins, colicins) as well as endotoxins (for which a number of test methods are prescribed in the Ph.Eur.)

Current methods of control of bioburden are based on the control of the planktonic organisms present within the system, material or product being tested. Biofilms are typically sessile (attached or fixed) but can also exist in a free flowing form for example during detachment. They can be difficult to identify within a system / process as their presence is usually relatively unknown until such time as an out of specification result occurs. This is because contamination of the water, where part of the biofilm has broken away, may be sporadic and random and therefore not easily detected using “grab” sample techniques.

Therefore measures should be taken by manufacturers to firstly, put in place scientifically justified mechanisms for maintaining biofilm control over such systems and processes, and prevent the further formation of such biofilms following proven methods for cleaning and sanitisation.

2. What approach should be taken to maintain control over systems which can be affected by biofilms?

A control strategy should be developed to assess the risks associated with the current manufacturing processes and to determine acceptability of existing control measures. The effectiveness of the sampling and testing regimes employed at the site should also be critically assessed in conjunction with the development of a control strategy.

3. What is a control strategy in the context of biofilm and contamination control?

A control strategy should take account of the design of the process, the mechanisms required to be put in place to control and ultimately prevent or minimise the risk of contamination.

Such a strategy requires the following thorough process knowledge and understanding taking account of all aspects of contamination control and prevention, including:

- Design – plant, process (the water system should be specifically designed to avoid dead legs, allow full drainage and minimise roughness)
- Control – including in-process controls
- Monitoring systems
- Prevention – Investigations / CAPA /root cause determination. Robust investigational tools are required
- Raw Materials
- Preventative maintenance – Maintaining equipment and premises to a standard that will not add significant risk from a contamination view point
- Equipment and facilities
- Process qualification
- Personnel
- Utilities
- Cleaning / sanitisation

Contamination control and steps taken to minimise the risk of contamination are a series of successive linked events / measures. These are typically assessed, controlled and monitored in isolation. Quality Risk Management tools along with scientific judgement can be applied in determining critical control points.

A contamination control strategy would integrate all of these measures to ensure a more comprehensive approach is taken with respect to prevention and control of microbiological contamination.

Such a strategy should lead to the introduction of a control programme which is an iterative process taking into account all information throughout the lifecycle of the products and processes.

4. If a biofilm exists what steps can be taken to eradicate or remove it?

The approach is both chemical and physical removal. When sanitising systems in this manner it is important to ensure that the systems are in recirculation mode and the sanitising agents utilised are not introduced into a system and left to exert their mode of action in a passive mechanism. Any approach to biofilm removal needs to be an active in operational strategy.

Use of chemical sanitising agents should be incorporated into a control strategy. While the utilisation of a hot water flush through systems is considered somewhat acceptable in order to minimise the planktonic contaminants existing within a system, it is known not have a significant effect on biofilms, which typically do not exist in a planktonic form, but usually in a sessile or attached form.

The ideal mode of action of chemical sanitising agents in the context of biofilm is to both penetrate and provide the appropriate kill to the organisms in question.

Appropriate removal of cellular debris should also be considered, as excessive debris can result in increased levels of endotoxin / exotoxin etc. existing within the system.

Frequent, rotation of disinfectants & detergents and inclusion of sporicidal agents should be considered as part of a robust strategy.

It should be noted that once a biofilm has been established it may be difficult to remove even using the methods above. Any biofilm removal should be followed by a period of intense monitoring before returning the system to use to ensure that the biofilm has been effectively removed.

A robust preventative maintenance programme is essential in order to maintain equipment and premises to a standard that will not add significant risk from a contamination viewpoint. Consider regular inspection of utilities, process equipment and transfer lines for obvious signs of deterioration – O-rings, gaskets, seals – regular inspection and replacement.

5. What specific agents can be used as part of a control strategy?

Examples include Sodium Hypochlorite, Hydrogen Peroxide / Peracetic acid solutions. Appropriate contact times need to be established.

Ozonation should also be considered for water systems.

Use of high temperature or steam sanitisation where possible should also be considered.

A singular approach to sanitisation is not an acceptable approach in order to minimise the risks of biofilm formation. In that regard an approach that utilises a minimum of a double-edged approach should be considered, e.g. high temperature in conjunction with a chemical sanitisation at a set frequency based on risk assessment.

6. Are there any additional measures which should be considered in order to increase the probability of detecting the presence of biofilms?

A robust sampling plan is a requirement. Such a sampling plan forms part of the control strategy employed to minimise such risks of biofilm and general contamination issues. Each potential source of contamination should be incorporated into such a sampling regime. The effectiveness of an environmental monitoring programme should be formally assessed at minimum on an annual basis.

Sampling programmes for water systems should take account of the quality of the water supply to the system as well as assessing points throughout water generation. User points should be tested each day of use in order to provide additional assurance of the quality of water utilised in the manufacturing processes.

Routine identification of contaminants isolated during monitoring activities is critical in order to ascertain if there is any shift or change in the flora present within a facility or if certain specific species become more prevalent.

Use of more sensitive endotoxin detection methods should also be taken into account. Alert limits should be set based on the capability of the system and any change or adverse trend should be appropriately investigated.

The frequency of trend analysis and use of trend data is critical.

Taking into account the speed at which organisms can proliferate, the use of rapid microbiological test methods and systems should be employed in order to improve or increase the probability of early detection and allow timely action to be taken.