Disinfection of an RO System Clearing the Issues

The proper disinfection of reverse osmosis (RO) systems is a difficult regime to recommend on a general basis because system designs can vary widely from unit to unit. However, as a steadfast rule, the monitoring and validation of the entire hemodialysis system--from RO to dialysis machine--is critical to the disinfection process. As such, bacterial monitoring for the purpose of validating frequency and efficacy of disinfection is crucial and should be performed correctly and routinely. Strategies for controlling bacterial colonization include regular, simultaneous disinfection of the entire RO unit, the delivery loop, and the hemodialysis machines, as well as limiting "downtime" and ensuring that the system and distribution piping are well designed in the first place. Many different disinfectants exist and may be appropriate for RO systems. It is important to vary the disinfectants used in order to obtain different results such as biofilm removal and/or reduction of specific microorganisms. It is also important to remember that just because a disinfection routine has worked well in the past does not mean it will continue to do so.

Disinfection of an RO system is one of those nebulous areas that is encountered in the dialysis setting. There are simply too many variables that can affect the outcome. This is because of the variances among facilities regarding system design, distribution piping, source waters, and basic philosophies. Also, there are not any black-and-white, "here's-how-you-do-it" easy answers. Even the related standards put forth by the Association for Advancement of Medical Instrumentation (AAMI) do not provide many specifics; in essence, as long as your unit meets the performance criteria and follows the manufacturer's recommendations, you are in compliance.

The AAMI guidelines further state that it is the responsibility of the RO system manufacturer to recommend a method of disinfection that will meet the standard, and that it is the user's responsibility to monitor and validate the process. With that in mind, this article will attempt to clear up some of the confusion surrounding disinfection of an RO system.

AAMI AND FDA RECOMMENDATIONS

As put forth in the AAMI standar, there are three levels of disinfection: high, intermediate, and low. High-level disinfection will eradicate all living organisms, with the exception of spores. Low-level disinfection or sanitization will kill many but not all microorganisms and is used in those siturationswhere reducing organisms to levels within the AAMI standard is deemed adequate and safe.(1) Intermediate-level disinfection is somewhere in between the two and, for the purposes of this article, will not be addressed further.

AAMI and FDA standards state that 200 cfu/ml (colony forming units/ml) is the maximum bacterial concentration allowable in the product water used for dialysis purposes.(2,3) The AAMI standard also expounds that the total microbial count in proportioned dialysate should not exceed 2,000 cfu/ml.(2) Additionally, according to the standards, voluntary endotoxin (lipopolysaccharide) levels in the water used for reuse should not exceed 1 ng/ml or 5 EU (endotoxin units) as demonstrated by the Limulus amebocyte lysate (LAL) assay.(3,4)

AAMI recommends that bacterial monitoring should be performed once a moth on the RO product water and dialysate. More frequent bacterial monitoring should be performed if the system is opened for any reason, if a new piece of equipment has been incorporated, or if the results of the water or dialysate cultures exhibit higher than allowable counts.

If the water used for preparing dialysate and or reuse purposes, or the dialysate itself, exceeds the allowable limit for bacteria and endotoxins, the dialysis patient can exhibit clinical signs such as shaking chills, fever, hypotension, myalgias, nausea and vomiting (pyrogenic reactions), and possibility sepis.(5) The onset of these symptoms usually occurs one to two hours after initiation of the treatment.(6)

BACTERIAL SAMPLING AND ASSAYING TECHNIQUES

It is not uncommon for dialysis units to receive inaccurate information from their laboratory regarding bacteria cultures. Therefore, before taking action, you need to be certain that a problem actually exists in the first place. To do so, it is necessary to examine your bacterial sampling and analyzing techniques, as well as the disinfection process. The following suggestions are offered to help facilitate that process.

When you collect your samples, wear long sleeves and gloves. This will help prevent skin bacteria from contaminating the samples. Also, do not draw the specimens from a silastic or silicone type hose, as many bacteria can colonize in this stagnate portion of tubing. Allow the sample port to flush for at least 30 seconds before collecting. Cleaning with Betadine is optional.(6) The "samples must be assayed within 30 minutes of collection, or immediately stored at 5 degree C and assayed within 24 hours of collection."(2) If the samples are allowed to stand, any bacteria present will proliferate, resulting in a false-positive result.

The laboratory should be instructed to do a standard plate count of viable microorganisms via a spread plate or membrane filter technique, including commercial dip test devices. The calibrated loop method which is used for urine culturing cannot be used for water or dialysate sampling as this is not a sensitive enough test and the minimum count is 1,000. If you frequently see results that have many zeros, be suspicious that the calibrated loop method is being used. And if you often have "no growth" test returns, investigate! Many times, the lab considers a low bacteria count as nothing more than contamination and therefore does not report it. Additionally, do not accept "negative" or "positive" as a result. Negative simply means that the count is less than 200 cfu/ml, and a positive report tells you little more than you are at or above 200 cfu/ml and beyond the AAMI maximum limit.(6)

"Culture media should be tryptic soy agar or equivalent."(2) Blood agar should be avoided because it is too nutrient rich for the waterborne bacteria and actually kills these microorganisms. Dialysate samples should only be grown on tryptic soy agar because the salt loving bacteria do well with this medium.(6)

The incubation temperature should be between 35-37 degrees C, and the colonies should be counted after a 48 hour period. (2) Many laboratories incubate at the lower 35 degree C temperature instead of 37 degree C, so the AAMI standard was recently changed to accommodate that fact.

Lastly, when you receive your valuable lab slips, do not tame them away in a notebook. This makes it difficult to track the trends in your dialysis equipment. Rather, make a spreadsheet that contains all the stations and numbered sample ports where the microbial specimens were drawn, including on it all the months of the year and the results in written or graph format. In other words, perform trend analysis.(6)

As more people turn to bottled water for a clean and safe alternative to drinking tap or well water, bottled-water suppliers will have to look harder for water sources free from external contamination. Meanwhile, ozone continues to offer a safe, reliable and cost-efficient solution for treating a wide variety of water treatment problems.

STRATEGIES FOR CONTROL OF BACTERIA

The goal of disinfection is to prevent and/or control the colonization of microorganisms, and a well designed water purification system and delivery loop is the first step in achieving this prevention.

In order to prevent stagnation--a cause of bacterial grrowth--the distribution piping in direct-feed systems (those with no storage tanks) must have a velocity of at least 1.5 ft/sec, and in indirect feed systems (those with storage tanks present), at least 3 ft/sec. It should be a continuous fast flowing loop design with no dead ends, rough joints, or unused branches in the piping.(7) The connections linking the product water to the dialysis machine should be accessible, minimal in length, and have a positive shutoff valve to prevent air from being drawn up into the loop. If a storage tank is present, it should be small in size in order to produce turbulent flows. It should also have a dish or conical shaped bottom for complete drainage, and have an airtight lid with a bacteria filter.(8) The incorporation of an internal spray mechanism will also prevent water from stagnating on the exposed surfaces.

The best strategy for a unit to use is to properly disinfect the entire hemodialysis system--from the water treatment components to the hemodialysis machines--on a routine basis according to the manufacturers' guidelines and based on routine bacterial monitoring.(9) This approach will bring about disinfection of a frequently forgotten area--the inlet water line to the dialysis machine.

Many facilities disinfect their central RO systems on a monthly basis and their portable RO systems once a week. When bacterial problems do arise, they do not wait for bacteria levels to increase to 200 cfu/ml; rather, they aggressively disinfect the entire system at much lower colony counts, typically anywhere from 20-50 cfu/ml. Otherwise, once the result is above the AAMI limit, the dialysis facility is out of compliance and the RO unit should not be used until the problem is rectified.

Some facilities draw bacteria samples more frequently than once a month, usually every 1-2 weeks. Remember, the AAMI guidelines are minimum standards, and by culturing more often, better control of the entire hemodialysis system is maintained.

In deciding how often to disinfect the RO system, remember that the RO water bacteria samples need to be drawn at the worst case scenario. Otherwise, how would you know that you only need to disinfect the RO system, say, once a month if your unit is culturing after the monthly disinfection? Also remember that this procedure only proves the efficacy of the sanitization process; it does not show whether the frequency of disinfection is adequate. Therefore, draw your culture samples before you disinfect the water purification system and hemodialysis machines. This will demonstrate whether your procedures are, in fact, sufficient.

Regarding which disinfectant to use, it is best, in theory, to alternate between different disinfectants in case a resistant strain of bacteria develops. A better reason for opting to use different chemical agents is for their individual characteristics. For example, one may be more aggressive at attacking biofilm--a gelatinous material secreted by bacteria in order to protect themselves from chemicals and to create an endless food supply--then another. Biofilm is very tenacious and difficult to remove once implanted. Another key piece of information to remember is to never mix disinfectants. And it should be noted that the laws in different states vary regarding the disposal of various disinfectants; therefore, check the laws in your state before adding a particular disinfectant to your regimen.

It has been shown that systems utilizing bolus-type injection sites for disinfectants do not thoroughly mix or assure the proper strength of disinfectant, resulting in inadequate disinfection of the system.(9) It is best, therefore, to premix the disinfectant to the proper strength and volume before introducing it into the system.

Regarding the RO membranes, a clean RO membrane that is free of debris will aid in the control of microbial growth in the system. RO membranes should be cleaned with both a high pH cleaner (to remove silt and organic material) and a low pH cleaner (to remove mineral scale) on at least a quarterly basis. This frequency, however, is dependent on the source water and on how efficiently the pretreatment is sized and working.

RO membranes are capable of removing up to 99.9% plus of bacteria, viruses, and endotoxins. However if the integrity of the RO membrane is broken with even a pinhole size tear, many microorganisms will be allowed to pass through. As a rule, RO membranes in large central systems can be expected to last 3-5 years, and the premature failure of RO membranes can be attributed largely to inadequate pretreatment. Therefore, assure that there is proper pretreatment, such as a carbon unit and a water softener placed before the RO unit, and replace the RO membranes when necessary.

Perhaps the largest culprit in the proliferation of bacteria in an RO system is "downtime". While it is a common practice to turn off the RO system when not in operation, it should be emphasized that stagnate water grows microbes. Some facilities recirculate the RO loop if a storage tank is present. Others do not because the water may become too warm and grow even more bacteria. If a system is left idle for more than 24-48 hours, it should be stored in disinfectant if left non-operational for long periods.

DISINFECTANTS

Always check with the manufacturer of the RO system before using a disinfecting agent that is not listed in the operator's manual, and always follow instructions thoroughly. Unfortunately, shortcuts often prove not to be so short in the long run.

When changing any disinfection regimen, such as using a different disinfectant, it is extremely important to validate the efficacy of the new regimen. Cultures should be drawn pre and post disinfection, and should be taken on a weekly basis for at least 2 months. It is important to establish a pattern of acceptable performance before backing off to less frequent bacterial testing.

It is especially difficult to recommend minimum dwell times for disinfectants because individual circumstances can vary so widely. However, keep in mind that longer contact times will kill more bacteria than will shorter exposure times.

Acetic acid and peroxide based compounds (Renalin or Minncare): Assure that the RO system is compatible with Renalin/Minncare. Units containing mixed metal or brass RO pumps and fittings are not Renalin/Minncare compatible. A 1% dilution is required for disinfection of thin film (TF) RO membranes. A lower concentration may be necessary with cellulose acetate (CA) membranes and is dependent upon the final pH. With a long enough exposure time, Renalin/Minncare will attack the protective biofilm layer and has good vapor phase activity.

Renalin/Minncare is a powerful oxidant, and if the mixture is higher than 1%, the RO membranes may be damaged. It is a common practice to consider the complete volume of the RO and distribution line when calculating disinfectant dilutions. This, however, is not appropriate for Renalin/Minncare use because the initial strength that the RO membrane experiences should not exceed 1%. The minimum dwell time of 36 minutes will result in a 6 log reduction. Renalin/Minncare should not dwell in an RO system for more than 12 hours; otherwise, permanent damage to the RO membrane will ensure.

It is recommended that the RO membranes be cleaned with the low pH cleaner that will remove mineral scale deposits before introducing the disinfectant into the system. This is especially true in areas that have high levels of iron and other transition metals in the RO feedwater, as Renalin/Minncare will react with these metals on the RO membrane and may cause degradation. It should also be noted that Actril is not recommended for use in RO systems; it is specifically made for dialysis machines, and the proportion of peroxide is to aggressive toward the RO membranes.

Formaldehyde or Formalin:
Many dialysis units are moving away from using formaldehyde because of exposure toxicity and intense OSHA regulations. For routine disinfection, a 1-2% solution is recommended. For aggressive disinfection, a 3-4% solution is recommended. Formaldehyde is compatible with all systems and has excellent vapor phase activity; it does not, however, attack the biofilm layer. A minimum dwell time of 2 hours for 4% formaldehyde may be effective, but it is best if the formaldehyde is left in the system overnight or longer. Formaldehyde may be used to store equipment for up to one year.

Bleach or Sodium Hypochlorite:
Never use bleach in an RO system containing thin film RO membranes. CA membranes are more chlorine-tolerant but have their individual limitations. Bleach is very aggressive toward biofilm when used to disinfect the distribution loop. A concentration of 500 parts per million, or approximately 1:100 solution, of common household strength bleach is an effective disinfectant. As a caveat, it should be remembered that bleach is very corrosive to the internal parts of the distribution loop, and dialysis machines and should be rinsed out within 30 minutes or less. Always use high-purity bleach. Never use the "off" brands because they may contain impurities that lodge in the system.

Gluteraldehyde (Nephrex):
Gluteraldehyde is not recommended for use in RO systems. It does not pass through the RO membrane in high enough concentrations to deem the system disinfected.

Sodium Metabisulfite:
Sodium metabisulfite is a bacteriostatic agent. It does not kill the bacteria but simply prevents them from replicating. The recommended long term storage dose is 0.1%. For short term "disinfection", a 1% concentration with a dwell time of 30 minutes or less is recommended. Sodium metabisulfite will degrade thin film RO membranes in higher concentrations or with longer dwell times. As with ingested sulfites in wine and salad, some people have severe reactions to sulfites. Therefore be extremely careful if you have a known sensitivity.

Low pH Cleaning Agents (Biosan):
Biosan is not listed as a disinfectant. It is a mineral scale deposit cleaner that has good biocidal activity. When used alternately with a disinfectant, it has been shown to prevent growth of bacteria in RO systems. It is recommended to use a 1:10 concentration of Biosan for a minimum of 30 minutes. RO systems with tin film RO membranes may be stored for up to 2 weeks in Biosan. With CA membranes, exposure should not exceed 3 hours.

HSI and NaOH:
At least one manufacturer suggests the use of these products as alternatives for the control of bacteria. Like Biosan, these are also considered cleaners, not disinfectants, and should be used in conjunction with a disinfection regimen. The hydrochloric acid at a pH of 2 should dwell for 30 minutes, and then, once thoroughly rinsed out, the sodium hydroxide at a pH of 11 should be introduced and left to dwell for 30 minutes.

Ozone:
Ozone is considered experimental at this point, but there are some dialysis facilities that have had good success with its use. Ozone has been well accepted in other fields that require high purity water, such as the pharmaceutical and beverage industries. Ozone is a powerful oxidant and is extremely degrading to RO membranes and other components. Therefore, it is used in the distribution piping alone, and all materials in the piping need to be ozone compatible. Ozone eradicates microorganisms and their byproducts, such as endotoxin, and breaks them down to carbon dioxide and water.

The half-life of ozone is 30 minutes in water and 24 hours in air. Their permissible exposure limit (PEL) in the atmosphere for ozone is 0.1ppm over an 8 hour time waited average (TWA). In other words, as long as the exposure averages out to 0.1 ppm at any given time in an 8 hour period, the person may be exposed to higher levels, but for shorter intervals. The effective amount used in RO distribution piping has been well under the PEL. Ozone is immediately broken down with exposure to UV light. It will also degrade quickly on its own.

Iodine:
Iodine is added to the purified water via an iodinator so that a steady concentration of iodine is achieved. Iodine has also been added pre RO to prevent bacterial degradation of CA membranes. The contact times and concentrations for hemodialysis applications are not well established. The iodine must be removed from the water before it reaches the hemodialyzer, and this can be accomplished through the use of a bed of granular activated carbon or a deionizer. However, because carbon and the deionizing resin are conducive environments for bacterial growth, they themselves will contaminate the water with bacteria, thereby defeating the original purpose of the iodine.

Ultraviolet (UV) Irradiation:
Ultraviolet light is produced by way of low pressure mercury vapor lamps that emit a bactericidal wavelength of 254 nm. Although UV destroys the bacteria as it passes by, it can cause endotoxin levels to increase in the product water. In order to be effective, the lamp must be replaced every 6 months to 1 year, and the protective quartz sleeve must be cleaned on a routine basis. UV resistant strains of bacteria can develop, making it necessary to use other means of disinfection.

SUMMARY

What works best for one dialysis unit may not work well for another. And it should always be remembered that just because a routine has worked well in the past does not mean it will continue to do so. All disinfection regimens should be well documented, monitored, and validated on a continuous basis.

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