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Bob Elms and Cindy Green

WATER SYSTEMS: THE BASICS

Part 1: Design as a Prelude to Validation

OVERVIEW

Water systems produce one of the most critical raw materials used in the manufacture of product. There is no system within a production facility that receives more focus in industrial journals and magazines or receives more attention during an FDA inspection than water systems. The impact of water on daily activities within the production facility is significant and therefore, the ability to receive and maintain high quality water on demand becomes essential.

The success (or failure) of products largely rests with the proper design, validation and continued maintenance of the selected and constructed water system. A successful design of a water system requires in-depth knowledge of the water quality requirements, determination of the critical operating parameters, identification of convenient delivery points, stringent maintenance procedures, and proper sampling and testing techniques. Part 1 of this two part series addresses the basics of water system design and identifies key information critical to the short and long term needs of a basic water system. Part 2 of the series will address the construction, validation, maintenance, and operation of a well-designed water system.

DEFINITIONS

There are a wide variety of systems available and creative ways in which to use these systems. Therefore, it is important that there is a clear understanding of the basic types of systems. The following definitions are generally accepted:

USP Purified Water: Water that is produced by distillation, reverse osmosis (RO), ion exchange or other means. The quality of water must result in conformance to USP specifications for purity.

Reverse Osmosis (RO) Water: Water that is produced by a reverse osmosis unit. This water passes through a system of membranes and is essentially demineralized.

Deionized (DI) Water: Water that is produced by passing treated water through a mixed bed or cation-anion exchange resin system.

Water for Injection (WFI): Water that is produced by RO or distillation, and conforms to the USP specifications for WFI.

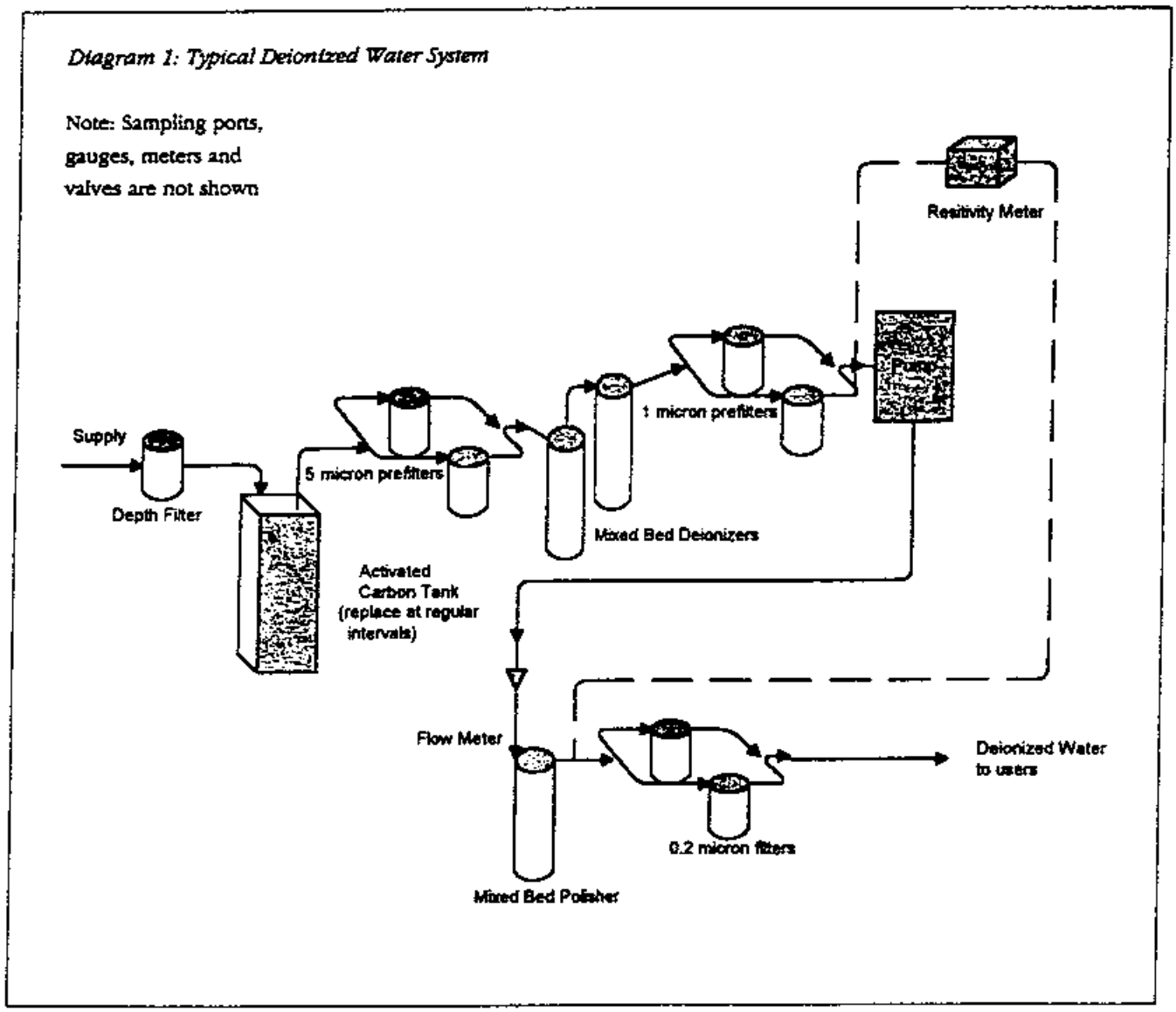
PRELIMINARY CONSIDERATIONS

Prior to the selection of the water system design, consideration must be given to the quality of the delivered water required for the current and proposed products to be manufactured. Over-design of the water system is not only initially expensive, but the ongoing maintenance can become unnecessarily burdensome with no return on the investment of time, money and personnel resources.

In order to determine the product sensitivity to water quality, several key issues need to be investigated. The system design project can begin by asking the following questions:

- Is there any evidence, documented or anecdotal, which suggests that the product formulation has the potential for adverse effects due to the water quality?
- What quality of water is used in the production of competitive or similar products? Is there an

Diagram 1: Typical Deiontzed Water System



industry standard that has become expected for use in the production of the current or proposed products? Does the water system need to provide deionized, purified or Water for Injection (WFI)?

• Is the product regulated by the Center for Devices and Radiological Health (CDRH), the Center for Biologics Evaluation and Research (CBER) or the Center for Drug Evaluation and Research (CDER)? What is the product classification? Is it a drug or is it a device?

• Does the product have preservatives? Is the product sterile? Is the product required to be pyrogen-free? What is the level of purity required for the product?

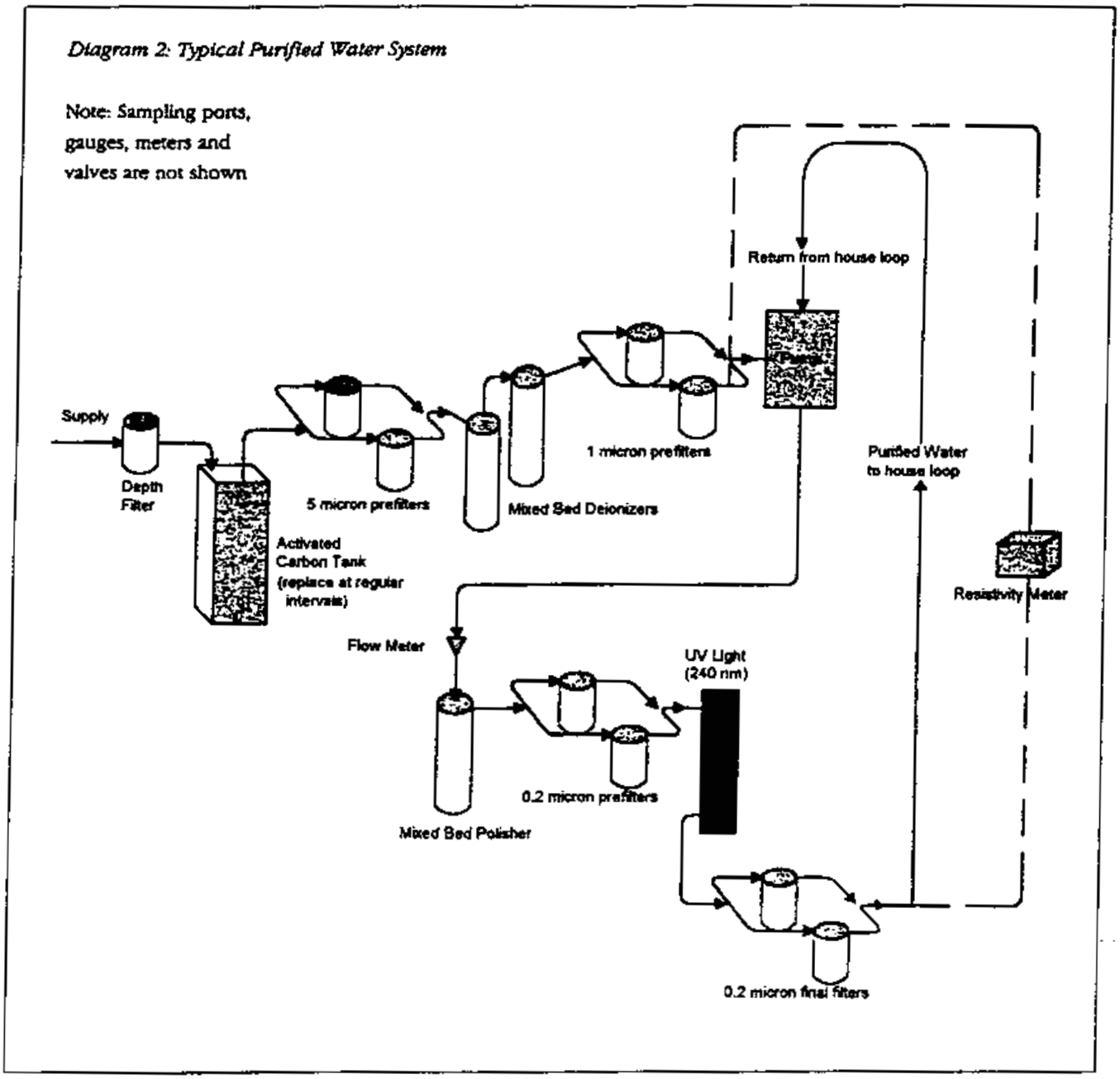
• Is water required as an ingredient in the product? Is water used in the processing? Is water only used for the cleaning of product contact vessels and equipment?

To address the capacity of the water system and the production needs, current and future, the following questions need to be addressed:

• What is the largest volume delivery currently needed? What is the largest volume needed for a distributed product during the first several years of market entry?

• What products are in development? What are the needs for these products?

Diagram 2: Typical Purified Water System



• Where will water be used in the facility? Do all points of use require the same quality of water? Do the volume needs vary between the use sites?

• Will the water be used directly from the use points or will it be stored in secondary containers? What is the length of time for storage and the expected volume to be stored?

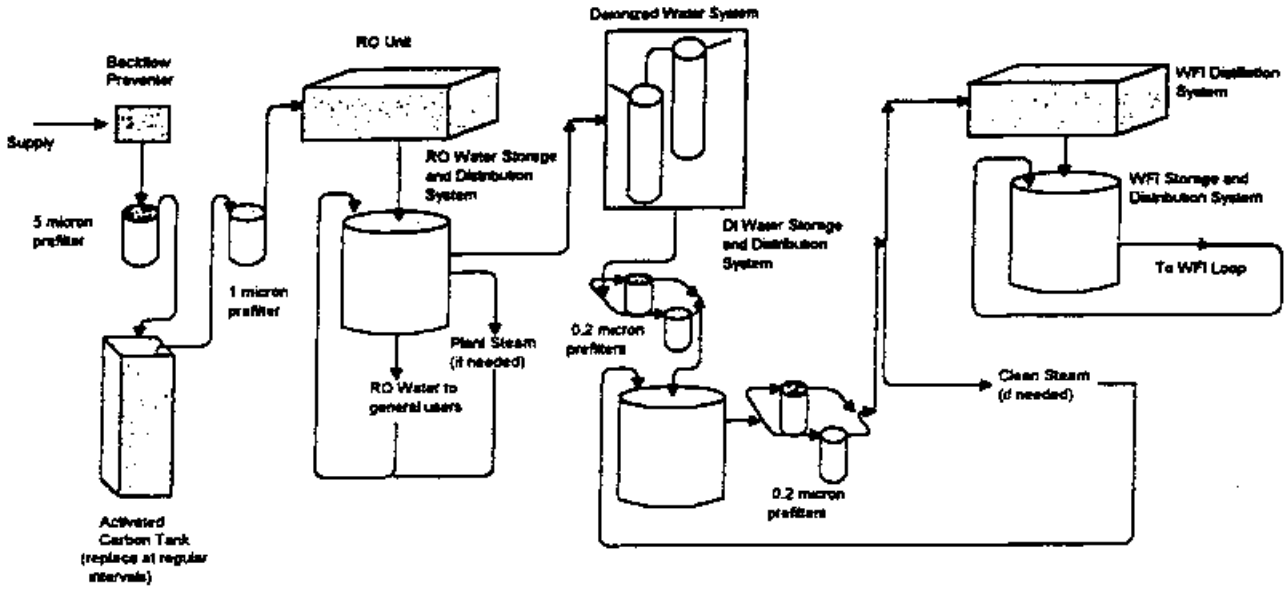
• Will the water be delivered to large volume use equipment, such as fermentation tanks or bulk reagent vessels? Will the water be used in the processing or cleaning of vials, large vessels, glassware and labware?

• Will the water be used for an autoclave, glass washer, or other equipment?

• What are the volume requirements for the equipment?

• Can water be collected and stored for "on demand use"? What is the regeneration time required to meet production needs?

Diagram 3: Overview of Production Sequence for Water for Injection



Considering the responses to the above questions, further evaluation must be made with respect to the material of construction. Materials used in the piping, storage tank, and on other water contact surfaces will depend on the quality of the water required. The expense of installing and documenting stainless steel versus plastic piping can be significant. In addition to the cost of construction, the required maintenance of the system over time should also be considered. The goal is to produce high quality water that consistently meets production requirements. If the system is expensive to maintain and resource demanding, a higher quality system design may be more cost effective in the long term. If the system design has the potential for contamination, it may also be more costly in the long term to install and maintain. The determination of the system cost versus the "product value" should be carefully evaluated in the final system design.

VENDOR SELECTION

The majority of newly founded companies do not have the benefit of having a facility engineer who is knowledgeable in water system design. It is therefore prudent for the design team to investigate the options that are available from vendors who specialize in the design and construction of water systems for FDA regulated industries. Much knowledge can be gained by having discussions with prospective designers and installers. With the basic needs identified, discussions will be significantly more beneficial and productive for both the company and the contractor. Identification of prospective vendors can be accomplished using several easy methods. First, and probably more effective, is to contact other similar industrial representatives. The network does work. Useful information may be gained by learning what worked (as well as what did not work) in the design and construction of other water systems used in other companies producing similar product types. Secondly, useful contacts can be made through conferences and meetings by visiting and discussing available services at the various vendor exhibits. In all cases, references should be obtained and investigated prior to serious discussions with prospective vendors. Vendor assessments should be made with respect to not only

their design input based on your projected needs, but also to the vendor's ability to meet expectations in the following areas:

- Final cost to original budget
- Timeliness and conformance to agreed upon time lines
- General responsiveness
- Knowledge of regulatory requirements
- Openness in discussion of the pros and cons of design options
- Agreement to provide justification for design preference
- Willingness to spend time in the plant with the users to assist in determining the system design needs
- Ease of start up and system operation
- Ability to provide required documentation timely
- Provision for maintenance contracts and troubleshooting support (Is there a local representative who will be on call in crisis situations?)
- Experience as a pharmaceutical vendor

Remember the contractor should be thoroughly familiar with the system operation. It is their primary business and they should be the experts. They should be able to easily address your questions and to provide the rationale for recommending one system over another.

SOURCE WATER CONSIDERATIONS

In the configuration of the system design, the source water quality must be considered. The local water company should be contacted by the design team and information obtained on the exact source of the water to be supplied to the facility. It should be determined if the water source has the potential to change depending on shortage or other factors. Seasonal variation should be discussed with respect to such things as algae blooms, increased silica during low reservoir states, etc. The water company should be able to provide periodic test reports and these should be compared to available water quality standards. Actual results should be reported and not a "pass or fail" qualifier. The water company should also be able to provide some in-sight as to what potential contaminants should be monitored and how often testing should be performed. The information gathered from the local water district should be added to the file and provided to the selected design and installation contractor. This information should also be reviewed during the development of the validation protocol, which will be addressed in Part 2.

The source water quality will have a definite effect on the design, selection and maintenance of the system components. The cost considerations for the maintenance of the components need to be factored into the system selection. In the event the source water is of particularly low quality, additional components may be needed or the preventative maintenance (for example, system component replacement and/or deionization resin regeneration) may need to be significantly increased.

PRELIMINARY DESIGN

Once some of the preliminary questions have been addressed and several prospective vendors have been identified, meetings should be held with each vendor to begin the process of designing the required system. There are numerous options and it is essential that system requirements are understood in order to provide specifications to the vendors. It is not unusual, however, for the details of the specifications to be incomplete at this stage, but it is important to work with the vendors and complete the design specifications through your discussions. The vendors will come prepared with a series of questions that will enable the design team to more accurately draft the design that will meet your requirements. Obviously, the more answers you have to their questions, the better suited the design will be. When dealing with several vendors it is extremely important to maintain the information shared with you during your discussions as confidential. It is

not acceptable to play one vendor against the other with the bidding process or design development. Each vendor should develop their own independent cost estimates with their own proposed system design.

Some of the potential vendors may be able to provide the piping for the loop and have the capability of installing the piping. If this is the case, discussions should be held at this same time to specify the required piping materials needed for construction as well as location of the points of use. If the vendor is not able to provide support for the loop, it will be beneficial to have separate meetings with the prospective piping vendors. Once the piping and water system contractors are identified, joint meetings with both groups should be held to coordinate the project. Consideration should be given to companies who have worked together successfully on other projects.

QUOTATIONS AND FINAL VENDOR SELECTION

Once the specifications have been developed for both the piping and the water system components, official bids should be provided to a minimum of three prospective contractors. The same information must be provided to all companies who are being asked to quote on the project. Upon receipt of the quotations, they need to be reviewed carefully for completeness and accuracy. The described options should be compared to the specifications and all components required should be listed with full descriptions and individual pricing.

In addition to the accuracy and completeness of the quotation, several other parameters should be considered along with the price for vendor selection. The questions that should be asked include:

- How soon did the vendor respond to the quotation?
- Was the sales person helpful and responsive?
- Did the sales person try to sell you a system that was different from the one you thought you needed?
- Were the questions asked during the design phase of the system meaningful?
- What is the service record for the system (obtain this information from the provided references)?
- Is there a local representative who can respond on 24 hours notice?
- What is the lead time for the system?
- Does the vendor have a reputation for "on time" installation and being responsive to system problems?

PLACING THE ORDER

Considering the specifications and agreed upon quotation, the purchase order can be prepared. It should be made very clear what you are getting for the quoted price, so it will be important to once again list the services and support items you anticipate receiving with the system. The following items need to be included:

- A complete drawing of the system that has been designed. Each component should be clearly identified with the manufacturer, model number and price along with other descriptive terms for clarity. This should include such items as deionization tanks, filters and housings, valves, sampling ports, gauges, UV lights, pumps, relief values and storage tanks.
- A full description of each component that will be included with the selected system. This should include material identification and a tracking record for all product contact surfaces.
- Written procedures on the vendor's preferred sanitization and start up procedures.
- Written procedures for calibration of the equipment.
- Written procedures for the service on the equipment and recommendations for a preferred service provider.
- Written operation procedures for the system along with specifications for such variables as pressure differentials between gauges, and resistivity.
- Recommendations for corrective action when the actual readings do not meet the established specifications.
- Written procedures for the installation of

new filters into existing housings, and regeneration of the deionization beds.

• Other supporting documentation, such as validation guidelines or recommendations, as well as other operational procedures.

• Spare parts list and recommended spare parts to stock.

It is important to negotiate for this documentation when the order is placed so the expectations are clearly understood by both parties. The vendor is the expert and has access to a significant quantity of reference documents as well as resource information based on historical experience.

If an arrangement can be made to have the drawings and component information provided early, a draft of the installation qualification section of the Water System Validation can be generated. With this section completed, it can be used when the technicians are actually installing the system components. This saves time, money and resources.

POTENTIAL PROBLEMS IN DESIGN SELECTION

When the time is not spent to properly plan, there can be significant problems which result. These include some of the following potential frustrations:

- The system is incapable of operating with extreme source water variation.
- The flow is inadequate to support the needs.
- The sampling ports are not adequate to provide a "clean" sample.
- The valves selected are not easily sanitized and allow contamination to occur.
- The sampling ports are not located to provide for ease of access and are not properly placed to appropriately monitor the system performance.
- There are not enough use points or too many use points.
- There are dead legs. Note: Dead legs should not exceed six pipe diameters in piping length.
- There is no flow during non-use periods.
- The system is not designed or constructed to provide the quality of water required for the particular product type.
- The carbon beds are not designed to provide for proper sanitization and to minimize bacterial growth.

CONCLUSION

Although time consuming and frustrating, the design planning for a water system will result in a high return for the time invested. Investigating what is actually needed, evaluating the pros and cons for the various options, talking to other users, and gaining in-depth knowledge of how water systems operate will have significant benefit. A well-designed system that meets the production needs, is well maintained by trained staff members, and monitored regularly (and correctly) will result in high quality and consistent water. Down time, troubleshooting and potential product problems will decrease cost and inefficiency plant wide. Spend the time upfront and design the system based on facts, not speculation.

In Part 2 or this two part series on "Water Systems: The Basics", the second article will address installation, start up, validation, sanitization, monitoring, sampling, testing, and maintenance of water systems. ■

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UTILITY QUALIFICATION - VOL II

Biodecontamination of Cleanrooms and Laboratories Using Gassing Systems | IVT

Tim Sandle

INTRODUCTION

Cleanrooms, laboratory areas, isolators and biosafety workspaces (microbiological safety cabinets) require a level of cleanliness and microbial control (achieved through disinfection) according to the intended use of the area. With cleanrooms this relates to microbial levels according to the class or grade of the room; with containment laboratories and biosafety cabinets this is with the intention of eliminating specific pathogens.

Decontamination can be undertaken using more traditional (and manually intensive) methods, such as spraying and wiping a suitable disinfectant onto a surface. Disadvantages with the manual approach include the need to remove residuals and the difficulty in ensuring coverage over an area (particularly in rooms). There are also concerns with the evaluation of the technique, which requires an extensive field trial to be conducted.

As an alternative, such areas can be effectively 'biodecontaminated' using gassing systems ('bio' denoting the elimination of microorganisms as opposed to chemical detoxification). There is considerable literature relating to the decontamination of isolators using gassing systems and the aim is not to repeat this here; instead the focus of the paper is with the decontamination by the use of gas of cleanrooms, containment laboratories and biosafety cabinets (1). Less has been written about the decontamination of these areas and the use of gassing systems to decontaminate these rooms and devices is increasing (or alternative chemical agents are being marketed). An alternative term to decontamination is fumigation, although this has closer connections with the use of formaldehyde and other poisonous fumigants and so 'decontamination' is the preferred term for this paper.

For cabinets, established approaches involve 'gassing' (a term which may refer to the use of a gas or to a vapor; the word vapor refers to a substance that in its natural state is a solid or liquid at room temperature, such as steam; whereas a gas in its natural state at room temperature would still be a gas, such a nitrogen) (2). Such methods have been used for the decontamination of microbiological safety ('biosafety') cabinets for decades, albeit more often using formaldehyde. The application of gassing systems to rooms (or suites of rooms) is a more recent development. Here hydrogen peroxide vapor is being used more frequently for decontamination. This includes hospital wards, containment laboratories and pharmaceutical facility cleanrooms (3).

Gaseous 'sterilants' are effective surface decontaminating agents in that

they will treat the outside of a device (or the primary packaging in which the device is held). The process is not typically referred to as 'sterilization' because unlike accepted forms of sterilization, like radiation or heat, the agent does not penetrate into the item being treated (4). Thus the terms 'decontamination' (or 'bio-decontamination') or sanitization are preferred. The key parameters affecting the effectivity of gas treatment are the active concentration, temperature, duration of exposure, and relative humidity (5).

In the past the primary method of decontaminating an area was through gas fumigation, using a substance like formaldehyde (formaldehyde is a gas which is soluble in water). This was despite few evaluation studies being conducted on the biocidal effectiveness of formaldehyde in the gaseous state, especially in consideration of the optimal parameters for microcidal kill (in terms of temperature and humidity). In addition, there are health and safety concerns associated with the use of formaldehyde, attributable to its toxicity and classification as a carcinogen. Alternative methods for gas decontamination of rooms are typically based on agents like hydrogen peroxide, chlorine dioxide and ozone.

The trends with the use gas or vapor to decontaminate an area are, firstly, the extension of the process from containment laboratories to cleanrooms; and, secondly, a reduction in the use of formaldehyde and a greater take-up of hydrogen peroxide or alternative agents. This paper assesses the current technologies and process steps required for the effective biodecontamination of cleanrooms and containment laboratories.

Formaldehyde

As indicated in the introduction, formaldehyde is an established fumigant and one traditionally associated with containment laboratories and biosafety cabinets. While it is unlikely that a cleanroom manager today would opt for formaldehyde fumigation, the use of the agent remains relatively widespread worldwide and this section focuses on the use of the chemical together with its advantages and disadvantages.

Formaldehyde is a naturally-occurring organic compound with the formula CH_2O. It is the simplest of the aldehydes. Formaldehyde is produced industrially by the catalytic oxidation of methanol. The production of formaldehyde was accidentally discovered by Alexander Mikhailovich Butlerov in 1859, with the official discovery being made in 1868 by A. W. Hofmann. From the late nineteenth century formaldehyde has become a major industrial product. One application is decontamination and formaldehyde vapor was the most common choice for decontaminating safety cabinets, and some rooms, at least until the 1990s. Formaldehyde remains in use, although many users have transitioned to systems that use hydrogen peroxide.

Formaldehyde vapor is theoretically effective biocidal agent (the degree to which it has been empirically tested is touched on below). To assess the effectiveness of formaldehyde biological indicators can be used, placed in appropriate locations. The chemical acts as an alkylating agent, inactivating microorganisms by reacting with carboxyl, amino, hydroxyl and sulphydral groups of proteins as well as the amino groups of nucleic acid bases. For formaldehyde to act to maximum efficacy it needs to reach the microbial cell. To do so formaldehyde must be able to dissolve at adequate concentrations in a film of moisture in the immediate vicinity of the microorganisms (6). Without such conditions, formaldehyde penetrates poorly.

To optimize the use of formaldehyde, the chemical is used in conditions, created by a proprietary fumigation device, which are both humid and warm (that is above 65% relative humidity and above 200°C). Alternatively, paraformaldehyde (the polymerization product of formaldehyde) can be vaporized in a pan on an electric element on the basis of 12 g per m^3 with simultaneous evaporation of 4 liters of water to supply the necessary humidity.

The main concern with the use of formaldehyde relates to the chemical having irritant and toxic properties. Use of the chemical carries a risk of respiratory damage and skin sensitization reactions. Formaldehyde can also react with chlorine to form bis- (chlormethyl) ether, which is a potent lung carcinogen. To safeguard against this, chlorine-containing disinfectants must be removed from areas prior to fumigation. An additional risk arises from formaldehyde being an explosion risk at a concentration of 7.75% (or higher) in dry air. This risk does not present itself at lower concentrations or in humid air.

These risks noted, formaldehyde remains in common use, especially in the laboratory setting where it is used for the decontamination of cabinets and rooms. These two applications are considered below.

Formaldehyde: decontamination of cabinets

Where formaldehyde is used for the decontamination of biosafety cabinets and equivalent devices the objective is to destroy any microorganisms that may have penetrated the High Efficiency Particulate Air (HEPA) filter. This is achieved through exposing of the downstream side of the HEPA filter and the ductwork to formaldehyde.

A generalized procedure to achieve this involves

the following steps (alternatively some cabinets have automatic fumigation cycles programmed into the controls and in these circumstances the manufacturers' instructions should be adhered to):

- Switch off the cabinet fans and wait at least 30 seconds.
- If the cabinet is a recirculation type, fit the fumigation adaptor kit to the discharge and position the other end to vent to atmosphere. A recirculation type of cabinet is ideal. Passive migration of the fumigant through the filter can occur but this does not lead to an optimal performance.
- Close the manual shut-off damper.
- Fill the vaporizer with the correct amount of formalin and screw on the aluminum cap - finger tight, having checked the gasket in the cap is undamaged. Place the vaporizer inside the cabinet.
- Fit the closure panel (or night door) and fully seal the front screen and closure panel with sealing tape to ensure there are no leaks.
- For safety reasons, place a notice on the front of the cabinet indicating fumigation is in progress.
- Switch the vaporizer on.
- After approximately 10 minutes (when half of the formalin will have been used) switch the cabinet fans on for 10 - 15 seconds.
- After a further 20 - 30 minutes switch the cabinet fans on again for 10 - 15 seconds.
- Leave the cabinet in this condition preferably overnight, but for a minimum of 6 hours (or preferably overnight).
- If the cabinet is a recirculation type check the exhaust of the fumigation adaptor kit is in a position to discharge safely and open the manual shut-off damper.
- Before venting the formaldehyde check that no people are in the vicinity of the exhaust outlet and that gas will not enter any open windows nearby.
- Exhaust the formaldehyde from the cabinet by switching on the fans and opening the closure panel/night door slightly (remove bung if fitted or crack open) until the majority of the formaldehyde has been exhausted. After about 10 minutes the night door may be removed completely.
- Any poly-formaldehyde residue in the vaporizer may be removed be heating with water containing a little mild detergent.
- Run the cabinet for at least a further 15-20 minutes to remove the last traces of formaldehyde.
- If the cabinet is a recirculation type the fumigation adaptor kit must be removed before the cabinet is used again.

It is especially important that following decontamination, the cabinet must be purged (aerated) of all residual formaldehyde.

Formaldehyde: decontamination of rooms

A similar controlled process is undertaken for the decontamination of cleanrooms and laboratory spaces. This should only be attempted if there is a means of exhausting the formaldehyde vapor from the room, via an extract system controlled from the outside of the room. The extract should be a total loss system with no possibility of formaldehyde being ducted to other areas. Before commencing fumigation the indoor space must be completely sealed to prevent escape of formaldehyde vapor into other areas.

Calculations need to be undertaken to estimate how long it will take to purge all the formaldehyde from the room after fumigation is complete. Such calculations should be based on room volume and the rate of air extraction through the particular exhaust system that will be used (7). The calculation needs to assess the initial formaldehyde concentration in the room and the target, which is the reduction of formaldehyde well below 2 parts per million (ppm) or 2.5 $mg.m^{-3}$. For this assessment the following formula is suitable:

$$\text{Time (minutes)} \times 25 = \text{room volume } (m^3) \,/\, \text{extraction rate } (m^3.min^{-1})$$

For the decontamination run it is typical to use 100 mL formalin plus 900 ml water per 28.3 m^3 (1000 ft^3) of room space.

As with the application of formaldehyde for the decontamination of cabinets, a series of steps can be followed for room decontamination:

- Switch off all forced air ventilation systems, extract systems and any fume cupboards and microbiological safety cabinets in the room. Additionally, deactivate fire alarm system smoke detectors.
- Seal up any external ventilation grilles.
- Confirm that the point from which the formaldehyde will be exhausted is free from obstruction.
- Place the appropriate quantities of formalin and water mixture into the heater unit. Activate the heater and leave the room immediately.
- Lock the door and effectively seal around the edges with tape.
- After a period of not less than 12 hours (for this reason, the procedure is best carried out overnight), the room must be well ventilated. Purge the space by using the remote switch to activate the extraction system (open air handling system dampers if necessary).

- Allow the room to purge for at least the time calculated as necessary to remove the formaldehyde.
- Check levels of residual formaldehyde in the room with suitable air monitoring equipment (such as a 'formaldemeter' or air sampling tubes).
- Responsible personnel should only enter the room if the level of formaldehyde is below 2ppm. It is recommended that personal protective equipment be worn.
- Check the room and all surfaces for formaldehyde residues and clean up as necessary.
- Remove all sealing materials.
- Other personnel can enter the room once formaldehyde levels are below 0.5 ppm.

Although formaldehyde remains in use in the laboratory setting, it is losing ground to hydrogen peroxide vapor. This is particular so with rooms that cannot be pre-cleaned prior to decontamination (such as areas that handle biohazards). Moreover, hydrogen peroxide vapor is the process of choice where the gaseous decontamination of cleanrooms within a GMP setting is required.

Hydrogen peroxide vapor

Hydrogen peroxide is clear, colorless, 'water-like' in appearance, and it has a characteristic pungent odor. Hydrogen peroxide vapor has a rapid antimicrobial efficacy, good material compatibility and, in comparison with formaldehyde, lower safety concerns. With microcidal efficacy, hydrogen peroxide is active against a wide range of organisms and this broad spectrum of activity is a product of the oxidizing capacity of the chemical. The chemical causes damage to cellular proteins, lipids and nucleic acids. With efficacy, hydrogen peroxide is more efficient in the gaseous form than in liquid phase.

The efficacy of hydrogen peroxide can be affected by the presence of both organic (such as proteins and lipids) and inorganic materials, which may reduce the penetration and activity of the agent. This is certainly the case in the presence of bacterial spores and *Mycobacterium species* (8), which are more resistant than other vegetative bacteria due to their unique lipophilic cell wall structure. The additional presence of organic materials can act as an additional shield to protect such microorganisms further. In addition there is some evidence to suggest that in, certain circumstances, microorganisms that produce catalase (a hydrogen peroxide degrading enzyme), such as species of *Streptococcus* (containing pyruvate oxidase), may be more resistant to the hydrogen peroxide treatment (9). Where these organisms are of concern (as might be evidence through environmental monitoring) this should be considered in a risk assessment.

An operational advantage with hydrogen peroxide is that it is compatible with a variety of different materials, such as plastics, metals, paintwork and electrical equipment. With safety although there are operational risks from the vapor, the risks are lower at the end of the process compared with formaldehyde for hydrogen peroxide is thermodynamically unstable and it decomposes to form water and oxygen. Safety concerns when using hydrogen peroxide include the vapor being an irritant of the eyes, mucous membranes and skin and it may cause lung irritation if inhaled. Additionally, skin contact with liquid hydrogen peroxide can cause temporary bleaching of the skin or redness and blisters if it is not immediately washed away.

There are two methods use to apply hydrogen peroxide for room or cabinet decontamination. These are:

- ***Aerosols.*** Here a commercial system produces a fine mist (particle sizes between 8 and 10 microns) of 5% hydrogen peroxide in air, with <50 ppm Silver ions, <50 ppm phosphoric acid, <1 ppm Arabica gum as catalysts. Over time, the aerosols collapse, the hydrogen peroxide reacts, and then degrades to safe conditions. There is little published validation of this method in hospital situations.
- ***Non-condensing vapor.*** With this approach, vapor is produced by a four-step sequence: an enclosed volume is first dehumidified. Then hydrogen peroxide (typically at a concentration of 35%) is vaporized under controlled conditions of temperature, humidity, and pressure so that there is no condensation. This state is maintained in the enclosure for a prolonged period during which super lethal concentrations of hydrogen peroxide are maintained in air for disinfection (10). In terms of microbial kill, this is the most important stage. Finally, the enclosure is purged with air (catalytic aeration) so that the concentration of hydrogen peroxide is below the product exposure limit (as an indicator of safety) (11).

Of these two approaches the second one is the more common. Non-condensing vaporised hydrogen peroxide is either referred to as VHP (which is trademarked) or as hydrogen peroxide vapor (HPV) in literature. For this reason HPV is used in the remainder of this paper to denote hydrogen peroxide vapor.

In addition to the different forms of generation, hydrogen peroxide vapor systems are often classified as "wet" or "dry" process. With this differentiation, hydrogen peroxide vapor can be introduced into a given area up to a certain concentration, dependent on the isolator temperature and humidity, to a

saturation level or dew point. If the concentration of hydrogen peroxide increases above this level it will condense onto the surfaces of the isolator (forming condensation or micro-condensation). In the case where micro-condensation is formed and maintained during the cycle, this is considered a "wet" process. If the vapor concentration is maintained below the dew point during the cycle this often referred to a "dry" process (although the vapor itself will not be completely "dry") (12). Although this description is useful in distinguishing between the two different processes, the term "wet" refers only to the fact that micro-condensation has occurred and this may not be visible to the naked eye. Different proprietary hydrogen peroxide generation systems claim to work on either "dry" or "wet" processes. Both, as far as this distinction matters, appear to be effective.
HPV is commonly used to decontaminate isolators. When used with barrier systems and rooms, HPV is a relatively rapid decontamination technology. Because the process, as discussed earlier, is a decontamination one, where critical surfaces are required (as would be the case with aseptic processing), these need to be separately sterilized.

Hydrogen peroxide process
A series of steps are required for decontamination using hydrogen peroxide. With this, vapor is produced through the use of a generator. Generators operate in a similar way, forming a closed loop with the area to be fumigated. Air is circulated through the generator in a four stage fumigation process (13). This process is:

- **Dehumidification**

With dehumidification, the humidity levels are reduced to a level below 40%. Some manufacturers of 'dry' HPV systems state that the dehumidification step is not required.

- **Conditioning**

For the conditioning stage, hydrogen peroxide gas (generated by vaporization of a 35% liquid hydrogen peroxide) is introduced to raise the concentration to a predetermined level. Sometimes this is called the 'gassing' phase.

- **Decontamination**

The decontamination phase is where the hydrogen peroxide concentration is maintained at a concentration below the condensation point, generally at 0.1-1.5mg/L at 25°C. Sometimes this step is referred to as the 'dwell' phase.

- **Aeration**

With aeration, the area is aerated to reduce peroxide concentrations to below 1 part per million. This is for occupational safety reasons. The longest phase in the cycle

While designing the control system for HPV cycle, for cleanrooms and isolators, the following factors should be considered (14):

1. The cycle should be designed in such a way that allows the complete airflow path to be subjected to decontamination, including the HEPA filters, valves, ducts and so forth.
2. The material properties of the load contents should be checked to ensure compatibility with the HPV.
3. With isolators, the room surrounding the isolator should be temperature controlled. Fluctuations in room temperature will cause fluctuations in the temperature of the isolator's exterior surface, leading to condensation on the isolator's interior surfaces.
4. The surface area of the load and the material is more important than the volume and contents of the load (this is essential for cycle development, as discussed below). Functionality must be designed in such a way that all moving parts inside the isolator are exposed to the gas. An intermittent movement of moving parts can be planned during a phase of cycle, if necessary. A glove-holding device and half-suit hangers should be used to keep gloves and half-suits from contacting any surfaces during decontamination.
5. The aeration cycle must be designed in such a way that residual concentration from the wrapped goods reduced to a safe level.
6. Provisions for holding the gloves in a position which means that the inner portion of the gloves and sleeves are exposed to the gas during the cycle.
7. The opening and closing of the tunnel gate should be automated as required during or after the cycle.
8. Chemical and biological indicators are required during validation and for annual re-qualification.

Cycle development
To demonstrate effectiveness a suitable HPV cycle should be developed and undergo validation. The effectiveness is demonstrated with the use of chemical indicators, to show vapor distribution, and biological indicators. For biological indicator selection, *Geobacillus stearothermophilus* spores are the most resistant to HPV (15). For the biological indicators a population of $>10^6$ is typical, with a resistance to hydrogen peroxide expressed as a D-value of between 1 and 2 minutes. With the configuration of the biological indicator, the spores are usually located on

a stainless steel concave disc, which is sealed inside a Tyvex pouch. The qualification should assess the influence of different construction material surfaces on inactivation of spores. Generally, plastic/elastomeric materials have generally higher resistance (D-values) compared with stainless steel and glass.

As with any sterilization or decontamination process, it is possible for failure to occur. To guard against this, cycles are designed for overkill. It is also possible for false positive results to be obtained. This can arise, for instance, if part of the pouch is not exposed correctly as with placing the biological indicator flat against a wall (16).

Comparing formaldehyde and hydrogen peroxide efficacy

The choice faced by users is between formaldehyde and hydrogen peroxide. In GMP processing, the choice is almost always towards hydrogen peroxide vapor. In laboratories, especially those used for containment, and with biosafety cabinets the selection is more even. Certainly older systems tend to use formaldehyde.

With formaldehyde, the effectiveness of the agent is based on historic assumption and there are few documented cases regarding its effectiveness. Certainly it is not easy to find data from confirmatory tests or comparison with HPV in this regard. The effectiveness of formaldehyde is considerably reduced if used at suboptimal ambient temperature or humidity. Moreover, temperature and relative humidity are rarely controlled during routine fumigations. HPV, in contrast, has been reported in a number of studies to be active against a wide range of organisms (17). However, as stated earlier, HPV is affected where surfaces have not been properly cleaned, especially where protein is present on surfaces.

Some of the advantages and disadvantages of formaldehyde and hydrogen peroxide are shown in Table 1, below.

Table 1: Comparison of formaldehyde and hydrogen peroxide for decontamination

Agent	Advantages	Disadvantages
Formaldehyde	• Long experience of successful use to decontaminate rooms and safety cabinets. • Inexpensive and easy to handle. • Simple to use and easy to detect. • Claimed broad spectrum efficacy. • Effective against M. tuberculosis.	• Slow acting, poor penetration. • Removal at end of decontamination • Strictly regulated in some countries. • Health effects include being toxic and carcinogenic • Reacts with chlorine to form bischloro-methyl ether. • Paraformaldehyde deposition.
Hydrogen peroxide	• Broad spectrum, rapid antimicro-bial. • Breaks down into non-toxic sub-stances. • Efficacy can be assessed using chemical and biological indica-tors.	• Efficacy affected by presence of organic • and inorganic materials (e.g. proteins and lipids) • Some catalase producing bacteria can show higher resistance. • Requires specialist equipment.

With both formaldehyde and HPV the efficacy is affected by ambient temperature and efficacy reduces at lower temperatures. Factors affecting the efficacy of both agents are:

- Composition of suspending fluid
- Microbial concentration
- Presence of protective agents

In selecting between the two agents, there is a substantially greater risk to safety from the use of formaldehyde compared to that with HPV. In relation to matters of GxP, the effectiveness of HPV can more easily be assessed through the use of chemical and biological indicators.

Alternative gassing agents

Although hydrogen peroxide and formaldehyde remain the predominant agents used for room and cabinet decontamination, there are alternative systems. This closing section of the paper considers

some of these.

Ethylene oxide

Ethylene oxide is an alky epoxide agent and it is deployed as a gaseous chemical sterilant. It is relatively stable and able to penetrate most polymeric materials. Biocidal activities are exhibited when the agent is dissolved in water. Here the concentrations required for biocidal effect tend to be quite high, in the order of 400-2,000 mg/L. An advantage is that many of the materials that function as a target for fumigation, such as metal and glass, do not absorb ethylene oxide. Consequently, little or no residue removal is required when using this fumigant. Additionally, the gas is ideal for thermo-labile devices as its application is performed at low temperatures (18).

Workplace exposure limits describe ethylene oxide as capable of causing cancer, therefore precautions are required. Ethylene oxide has a flammability range from 3-100% by volume in air; thus the agent should be supplied with inerting chemicals, designed to contain any explosive event.

Propylene oxide

Propylene oxide possesses many of the sterilant properties described for ethylene oxide, although a longer processing time is required. The use is generally confined to the food industry. The advantage is with the agent having narrower flammability.

Peracetic acid

Peracetic acid is an oxidizing agent. It can be used in the liquid form or in the vapor phase. Like hydrogen peroxide, the chemical is sporicidal. In the vapor phase peracetic acid displays an optimal activity at 80% relative humidity (RH). The agent has a slower decay rate in an open chamber than hydrogen peroxide. The decomposition by-products include acetic acid, hydrogen peroxide, water and oxygen. In terms of advantages, peracetic acid is not absorbed as easily as hydrogen peroxide by cellulose-based materials.

Chlorine dioxide

Chlorine dioxide is also an oxidizing agent and a sporicide. In the gaseous form it is relatively short-lived and used at 25-30°C and at a concentration of 10-50 mg/L, at 80% relative humidity. Chlorine dioxide has a typical 8 hour run time for a typical application. Applications are limited because the molecule has a damaging effect on some materials (19). The use of chlorine dioxide has been tested out in a number of hospital facilities. One study showed chlorine dioxide to exhibit effective kill of *Bacillus anthracis* spores, together with spores of *Bacillus atrophaeus* and vegetative cells of both *Francisella tularensis* and *Yersinia pestis* (20). Chlorine dioxide kills microorganisms by disrupting proteins, interfering with protein synthesis and membrane transport.

Ozone

Ozone is a natural form of activated oxygen and it is formed when oxygen is exposed to a high-energy field. It is a triatomic molecule (O_3), consisting of three oxygen atoms. Ozone is much less stable than oxygen (O_2), breaking down, with a half-life of about half an hour in the lower atmosphere, into oxygen. Ozone occurs naturally in the atmosphere and it is produced during lightning storms and continuously occurring in the stratosphere due to action of ultraviolet light.

As a sterilant, ozone is classed as an oxidizing agent, and it breaks down into oxygen molecules and oxygen atoms, which have high oxidation potential. Ozone acts on the microbial cell membrane and damages the membrane structure causing metabolism disruption. For secondary action, ozone infiltrates the cell membrane and destroys lipoprotein and lipopolysaccharide, changes permeability and causes cytolysis and cell death. As well as being effective against prokaryotic organisms, ozone is an effective protozoan cysticide (21).

Ozone is used across a variety of industrial settings to sterilize water, as well as a disinfectant for surfaces. However, there is little in the way of case studies to support its use for air disinfection. Effective applications have been reported by use at between 2 and 10% by weight. The case against consideration of ozone for the decontamination of containment areas is the fact it will harm and destroy materials used in containment facilities, especially when used at a high concentration.

Plasma

The technology for plasma generation and its known inactivation of microorganisms has been known since the 1950s, although commercial applications remain slow to emerge. Plasma is energetically distinguishable from solids, liquids and gases. Plasma can be produced by the action of very high temperatures or electric or magnetic fields, normally composed of clouds of ions, electrons and neutral species. With anti-microbial action, electrical discharge can generate free radicals and other potentially biologically active species from hydrogen peroxide.

Qualification

Whichever agent is used, the process used must be