Laboratory guide to **Water Purification**

An industry service publication

Introduction to Water Purification

About this guide

This booklet was developed to serve as a basic guide in the selection of water purification equipment. The information presented is "generic" in nature. That is, while experienced Labconco personnel have compiled this booklet, it is our intention to provide a non-biased review of water quality standards, common water contaminants and purification methods. The purpose of this booklet is to help you make an informed choice for your laboratory situation.

Water is a reagent that has often been taken for granted. It falls from the sky, it flows in the rivers, it is available at the tap at the turn of the faucet, we drink it, bathe in it — if it looks clear and we are accustomed to thinking of it as being pure. However, in the laboratory, potable water is often not pure enough.

It is common for analytical and experimental scientists to be concerned with elements and compounds in the parts per billion (ppb) and parts per trillion (ppt) range. Life science research is often very sensitive to many contaminants, especially heavy metals and dissolved organics. High performance liquid chromatography (HPLC) requires ultrapure water in many of its applications, including calibration of detector base lines and elution of reverse phase columns. Trace element analysis requires water that is free of the elements in question.

What kind of work are you doing with pure water?

How are you purifying your water now?

Is the water in your laboratory pure enough for the sensitivity of your analytical procedures?

Principles of Water Standards

Water Standards

In response to developments in scientific technique and technology and the increasing sensitivity of research, several professional organizations have established water quality standards. These groups include the American Chemical Society (ACS), the American Society for Testing and Materials (ASTM), and CLSI which are responsible for determining water quality measures for the clinical laboratory.

As an example, the CLSI has specified three types of water — I, II, III — and Special Purpose, which are listed below with their intended uses. Below are specific applications and their feedwater water requirements.

Type I - Test methods requiring minimal interference and maximum precision and accuracy:

- Atomic absorption
- Flame emission spectrometry
- Ligand assays
- Trace metals
- Enzymatic procedures sensitive to trace metals
- Electrophoretic procedures
- High sensitivity chromatographic procedures
- Fluorometric procedures
- Buffer solutions
- Standard solutions

Type II - Test methods in which the presence of bacteria can be tolerated or where requirements leading to the choice of Type I or Special Purpose waters does not apply:

- General reagents without preservatives
- Microbiology system (not to be sterilized)
- Stains and dyes for histology
- General reagents with preservatives
- Microbiology systems (to be sterilized)

Type III - General washing and feedwater for producing higher grade water, as well as bacteriological media preparation.

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Type I Water Quality Standards

	CLSI	ASTM
Resistivity[1], megohms-cm, at 25° C, minimum	10.0	18.0
Conductivity, microsiemens/cm, maximum	0.1	0.056
Silicate, mg/l, maximum	0.05	.003
Particulate matter, μm filter	0.22	0.2
Microorganisms, colony forming units per milliliter	10	see note 2

[1] Resistivity and conductivity of Type I water must be measured in-line. Measuring in a container will give inaccurate readings.

[2] For ASTM: Type IA water - 10/1000 ml
Type IB water - 10/100 ml
Type IC water - 100/10 ml

Contaminants and Water Testing

The four types of contaminants commonly found in water are:

- **Particulates**
- **Dissolved inorganics (solids and gases)**
- **Dissolved organics**
- **Microorganisms**

For a better idea of what these standards mean, let us examine the kinds of contaminants that may be found in water.

Water is an excellent solvent and the medium of most life processes on this planet. This explains why water is easily contaminated with many things that it encounters and why microorganisms can thrive in it. The aforementioned is why purifying water is a much more difficult procedure than it initially might seem to be.

Particulates include silt, plumbing pipe debris, and colloids. These suspended particles can plug filters, valves, lab tubing, reverse osmosis membranes and conductivity meters. Particulates are visible as cloudiness or turbidity, and are detected using filtration and gravimetric means, or microscopic methods. A 10 to 20 micron prefilter is often placed as the first component in a water purification system to filter out the larger particles. Smaller particles are removed subsequently by reverse osmosis, submicron filters and ultrafiltration membranes.

Dissolved inorganics ions such as calcium, magnesium, potassium, and sodium (these ions affected the hardness of water), gases such as carbon dioxide that ionize in water (carbon dioxide dissolves readily in water to make mildly acidic carbonic acid), silicates leached from sandy river beds or glass containers, ferric and ferrous ions from rusty iron pipes, chloride and fluoride ions from water treatment plants, phosphates from detergents, nitrates from fertilizers, and many others.

There are several tests for identifying specific dissolved inorganics. The simplest test is a direct measurement of electrical conductivity or resistivity. Most dissolved inorganics are either negatively charged (anions) or positively charged (cations), and will transmit a current when a voltage is applied to electrodes inserted in the water. The more ions present, the greater the conductivity, or the lower the resistivity of the sample water.

Conductivity is expressed in microsiemens/cm and is used to measure water with a large number of ions present. Resistivity is expressed in megohms-cm and is used in the measurement of water with few ions. Conductivity and resistivity are reciprocals of each other. Thus, at 25° C, 18.2 megohm water, which is the highest purity water obtainable with today's technology, also has a conductivity of 0.055 microsiemen/cm.

Resistivity	0.1	1.0	10.0	18.24	megohm-cm
Conductivity	10.0	1.0	0.1	0.055	microsiemens/cm

Does your analytical work require that your water be free of dissolved organics?

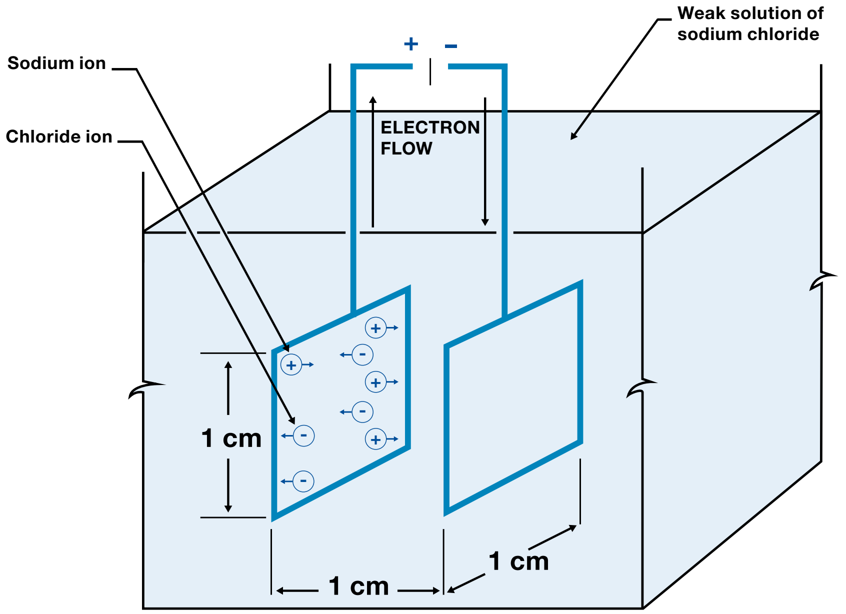
Dissolved organics may include pesticides, herbicides, gasoline, and decayed plant or animal tissue. Dissolved organics may also include the plasticizers leached out of plumbing lines, fittings and storage tanks.

Note the sources of the last organic contaminant — all are from improperly designed water purification systems. Thus, a water purification system must both remove the contaminants present in the feedwater, and be designed to minimize the addition of contaminants to the water.

The absence of dissolved organics is very important when performing analyses of organic substances in HPLC, gas chromatography, electrophoresis, and fluoroscopy, or in research involving tissue cultures.

There are several ways of measuring dissolved organic levels. The potassium permanganate (KMnO4) color retention time test is a qualitative organic test that may be used. The premise of this test is that the bright purple-colored potassium permanganate, a powerful oxidizing agent, will change color to clear if there are sufficient organics present in the water to be oxidized. The drawbacks of this test are that it is slow, it is not sensitive to very low levels of organics (that might still be too high for HPLC purposes), and it is not quantitative.

Total Organic Carbon (TOC) analyzers, which oxidize the organics and measure the CO2 liberated, are being used more and more to determine organic levels in Type I water due to their low-level detection sensitivities. A low TOC level is very important for HPLC users.



This figure shows the standard configuration of a conductivity cell and the movement of the anions (-) and cations (+) toward the charged poles.

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The challenges for an ultra-pure water purification system are to

- **remove the bacteria present in the feedwater**
- **prevent bacteria from entering the system and contaminating it**
- **ensure that no bacteria are in the product water**
- **inhibit bacterial growth through proper design and operation.**

How are doing HPLC?

Is it important to you to have very low levels of dissilved organics?

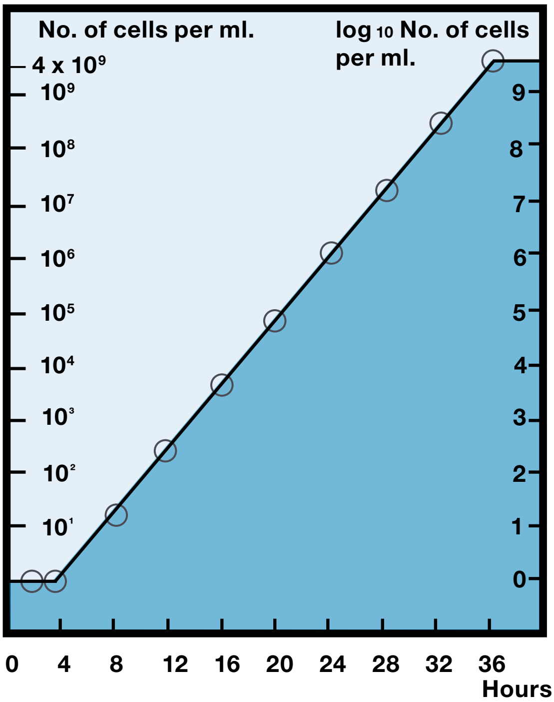
Microorganisms constitute another group of contaminants found in water. Surface water may contain a wide variety of microorganisms, including bacteria, protozoa, algae, amoebae, rotifers, diatoms and others. Since most laboratory water comes from municipal water treatment plants, which is extensively treated to remove microorganisms, the chief microbes of concern for water purification systems are pathogenic bacteria. A typical bacterial level for a potable laboratory water supply is one colony forming unit per milliliter (cfu/ml).

Bacteria are celled organisms that multiply at exponential rates, thrive in standing water, and may be present on many surfaces and in the air. Bacteria subsist on a variety of substrates in purified water including dissolved organics such as plasticizers and dissolved inorganics such as iron and sulfur.

Bacteria will enter an unprotected water purification system from the feedwater, breaks in the purification system, or through the dispenser. Once in the system, bacteria secrete a polymeric substance that adheres them to surfaces of storage tanks, deionization cartridges, plumbing, and hard-to-clean surfaces.

Bacteria are usually detected and enumerated by filtering the sample water through a 0.45 micron filter and culturing the filter on a suitable medium for several days.

Bacteria can be killed with disinfectants like hydrogen peroxide, hypochlorite, and formaldehyde. However, when bacteria die, their polymeric secretions and lipopolysaccharide cellular fragments remain and may be a source of contamination if not removed. These cellular fragments are typically pieces of cell wall from gram negative bacteria or liopolysaccharides When injected into a mammal, pyrogens cause a rise in body temperature. Thus pharmaceutical grade water must be pyrogen-free. Pyrogens also have a detrimental or lethal effect on tissue cultures.



A culture of bacteria (one organism per milliliter) in glucose medium after a lag of about 4 hours grows exponentially with a doubling time of one hour.

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Pyrogens are detected either by injecting the sample water into specially bred rabbits and monitoring them for a body temperature rise, or with the LAL (Limulus Amoebocyte Lysate) test, a sensitive test for very low concentrations of lipopolysaccharides.

Have you analyzed your feedwater and product water for bacterial and pyrogen levels?

Have you had problems with bacteria proliferating in your water purification system?

Do you require that your water be bacteria-free?

Do you require that your water be pyrogen-free?

Discussing the types of contaminants found in water leads us to a review of the ways that these contaminants may be removed from feedwater to produce ultrapure reagent quality water. A discussion of these technologies follows.

Eight different methods are commonly used to purify water. These are:

- **Distillation**
- **Deionization**
- **Reverse osmosis**
- **Activated carbon filtration**
- **Microporous filtration**
- **Ultrafiltration**
- **Ultraviolet oxidation**
- **Electrodialysis**

Water Purification Process Comparison

E = <u>Excellent</u> (capable of complete or near total removal)

G = <u>Good</u> (capable of removing large percentages)

P = <u>Poor</u> (little or no removal)

	Dissolved Solids	Dissolved Gases	Dissolved Organics	Particulates	Microorganisms	Pyrogens
DISTILLATION	E G(1)	P	G	E	E	E
DEIONIZATION	E	E	P	P	P	P
REVERSE OSMOSIS	G(2)	P	G		E	E
CARBON FILTRATION	P	P(3)	E G(4)	P	P	P
MICROPOROUS FILTRATION	P	P	P	E	E	P
ULTRAFILTRATION	P	P	G(5)	E	E	E
U.V. OXIDATION	P	P	E G(6)	P	G(7)	P

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(1) The resistivity of water purified by distillation is an order of magnitude less than that produced by deionization, due mainly to the presence of CO_2.

(2) The residual concentration of dissolved solids is dependent on the original concentration in the feedwater.

(3) Activated carbon will remove chlorine by chemisorption.

(4) Special grades of carbon are available which exhibit excellent trace organic removal capabilities.

(5) Ultrafiltration will remove organics based on molecular weight cutoff of ultrafilter membrane.

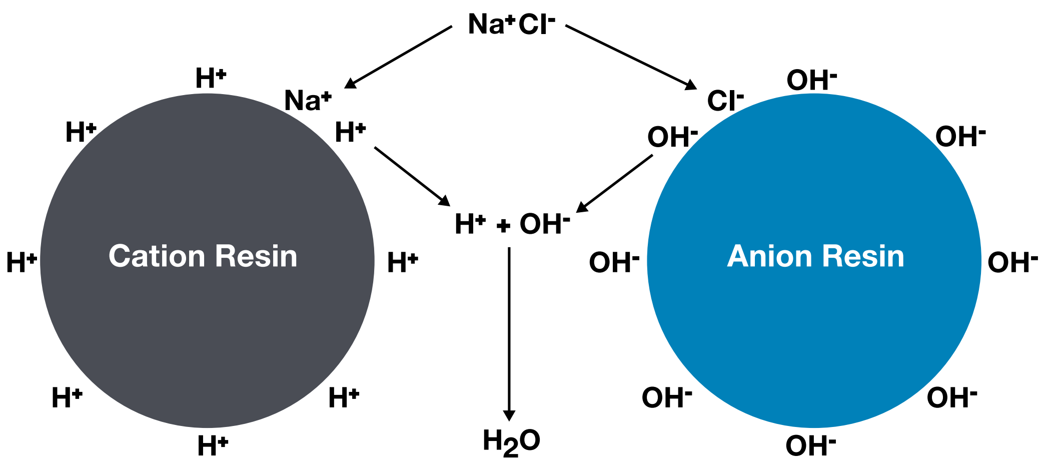
(6) Certain UV oxidation systems have been specifically designed to exhibit excellent trace organic removal capabilities. These are not to be confused with UV sterilizers.

(7) UV systems, while not physically removing bacteria, may have bacteriocidal or bacteriostatic capabilities limited by intensity, contact time and flow rate.

Distillation

Distillation has several positive features. The equipment is relatively inexpensive, no expendables other than replacement glassware and heating elements, and it produces water of good quality. Distillation typically produces water of Type II or III quality, with a resistivity of about 1.0 megaohm.

Distillation has several drawbacks, however, and because of these, is not as widely used as in the past. Distillation is not an on-demand process. Because of this aspect, a quantity of water must be distilled and stored for later use. If the storage container is not made of an inert material, ions or plasticizers will leach out of the water container and recontaminate the water. Bacteria are known to grow well in standing water. The bottles may be sterilized and the collected water autoclaved. However, once the bottle is opened, it is exposed to bacteria and contamination begins.



Anions and cations in the feedwater pass through the ion exchanger resins and replace the hydrogen and hydroxyl ions attached. The hydrogen and hydroxyl ions then combine to form pure water molecules.

Deionization has several advantages (over distillation) for the production of purified water. It is an on-demand process supplying purified water when needed. Nuclear grade deionization resin or polishing mixed bed resin removes almost all the ionic material in the water to a maximum resistivity of 18.2 megohm-cm (at 25° C).

Deionization alone, however, does not produce absolutely pure water. Tiny fragments of the ion exchange resin are washed out of the system during operation and stagnant water in the cartridges may allow excessive bacterial growth. Deionization also does not remove all dissolved organics from the feedwater, and in fact, dissolved organics can foul the ion exchange resin. Finally, deionization cartridges can be an expensive option for labs that choose to replace their cartridges rather than regenerate them.

There have been many attempts to overcome the shortcomings of deionization and distillation. In some setups, distillation has preceded deionization — the cartridges last much longer, but the problems of bacterial contamination remain.

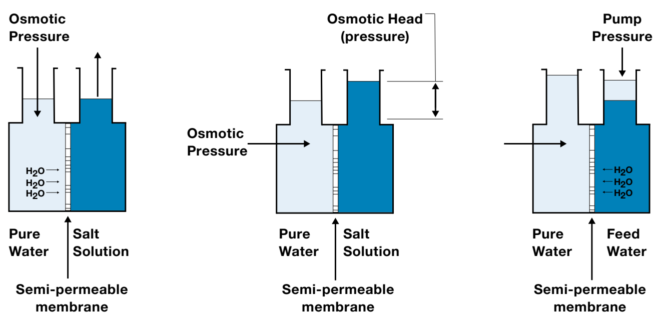
Reverse Osmosis is a process which overcomes many of the shortcomings of distillation and deionization.

Reverse osmosis can be explained better after understanding the natural process of osmosis. Osmosis is the movement of water across a semipermeable membrane from the less concentrated high water potential side to the more concentrated low water potential side. This movement continues until the concentrations reach equilibrium or the pressure on the more concentrated side becomes high enough to stop the flow. Osmosis is the natural process by which water is drawn into a plant's root, or moved from one cell to another in our bodies.

If a pressure greater than the osmotic pressure is applied to the more concentrated solution, using a high pressure pump, water molecules are pushed back across the membrane to the less concentrated side, yielding purified water. This is the process of reverse osmosis.

Reverse osmosis typically removes 90-99% of most contaminants. A table of reverse osmosis performance characteristics follows:

Because of its exceptional purifying efficiency, reverse osmosis is a very cost effective technology and is often used to pre-purify tap water for further purification by other technologies. Since reverse osmosis removes a high percentage of bacteria and pyrogens, it is often combined with ion exchange to significantly prolong the life of the deionization "polishing" cartridges. In addition, a system which allows dispensing of the reverse osmosis water gives a source of high quality pre-purified water, which is suitable for many routine laboratory purposes.



(a) OSMOTIC FLOW (b) OSMOTIC EQUILIBRIUM (c) REVERSE OSMOSIS

Contaminant	Removal Efficiency
Suspended solids	100%
Bacteria	99.5%
Viruses	99.5%
Pyrogens	99.5%
Organics, molecular weight > 250 Daltons	97-99.5%
Monovalent inorganics	94-96%
Divalent inorganics	96-98%
Trivalent inorganics	98-99%

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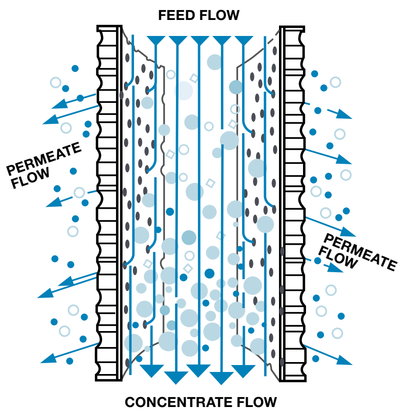
Activated Carbon Filtration removes chlorine by chemisorption and dissolved organics by adsorption and is often found at two places in a water purification system. Because thin film composite reverse osmosis membranes are sensitive to chlorine, and to a lesser degree, fouling from dissolved organics, activated carbon is often placed before the RO membrane to remove these contaminants.

A granular activated carbon filter is also often placed in the polishing loop of a water purification system to remove trace amounts of dissolved organics for water quality suitable for HPLC work.

Microprous Filtration or submicron filtration uses a membrane or hollow fiber with an absolute pore size of 0.2 micron that prevents any contaminant larger than 0.2 micron from passing through it. The submicron filters retain carbon from the carbon cartridge, resin fragments from the deionization cartridges and any bacteria that may have entered the system.

CLSI considers water to be particulate free when it has been passed through a 0.2 micron filter. Microporous membranes are generally considered to be indispensable elements of a water purification system, unless they are replaced by an ultrafilter.

Do you need laboratory water to be particulate free per Type I standards?



Crossflow filtration in an ultrafilter showing pyrogens and particles larger than 0.002 micron being retained, while only pure water molecules pass through the pores. Courtesy of OSMONICS, INC., Minnetonka, MN, USA.

Ultrafiltration uses a membrane very similar in design to reverse osmosis systems except that the ultrafilter's pores are slightly larger. The ultrafilter is used to remove pyrogens and other long chain organic molecules such as RNase from the purified water.

Since a high percentage of the water brought to the ultrafilter passes through it, it will eventually plug if not maintained. In a properly designed system, the ultrafilter is regularly and tangentially washed free of contaminants. With this type of design, ultrafiltration is an outstanding technology for ensuring very consistent ultrapure water quality.

Does your work require that you use pyrogen-freewater?

Ultraviolet or Photo Oxidation uses ultraviolet radiation at the biocidal wavelength of 254 nanometers to eliminate bacteria from the system. It also cleaves and ionizes certain organics at 185 nanometers for subsequent removal by the deionization and organic adsorption cartridges in the polishing loop.

Electrodialysis (ED) removes impurities from water using an electrical current to draw ionic contaminants through ion selective membranes (ion exchange resin in sheet form) and away from the purified water. Used occasionally to produce potable water from clean brackish feedwater, ED is cost competitive with reverse osmosis.

To produce laboratory grade water, however, ED has several drawbacks and, as such, is rarely used in lab settings. First, the contaminants ED can remove are limited. ED cannot remove contaminants such as certain organics, pyrogens and elemental metals which have weak or nonexistent surface charges because they are not attracted to the membranes. Second, ED requires a skilled operator and routine maintenance. Large molecules which bear a significant charge such as certain colloids and detergents can plug the membranes' pores, reducing their ionic transport ability and requiring frequent cleaning. During operation, ED liberates caustic soda which may cause scaling, and hydrogen gas which is potentially dangerous. Finally, ED is relatively expensive. As ionic contaminants are removed from the water, its electrical resistance increases, so that higher electrical current is required to continue the purification process. Purification beyond the potable level is considered uneconomical due to the increased electrical consumption. Component materials such as platinum and stainless steel are also expensive.

Glossary

Activated Carbon: A porous carbon material used for adsorption of organics and absorption of free chlorine.

Adsorption: The physical attraction and adherence of gas or liquid molecules to the surface of a solid.

Anion: A negatively charged particle or ion.

Carbon fines: Very small particles of carbon that may wash out of an activated carbon filter.

Cartridge: A prepacked method for housing the filtering components of a water purification system. Because of the modular design of filter elements placed in a cartridge, the changing of exhausted filters is greatly facilitated.

Cation: A positively charged particle or ion.

Chemisorption: The formation of bonds between the surface molecules of carbon and chlorine coming in contact with it.

Colloid: A stable dispersion of molecular aggregates in water that have a size ranging between one and two hundred millimicrons. Colloidal iron, aluminum, and silica are commonly found in water.

Concentrate: The reject water from a reverse osmosis membrane; called the concentrate because it contains a higher level of contaminants than the feedwater.

Conversion rate: A quantification of the relationship between the volume of feed and product water of a reverse osmosis membrane.

Deionization: The process of removing the charged constituents or ionizable salts (both organic and inorganic) from solution. A purification process that uses synthetic resins to accomplish the selective exchange of hydrogen or hydroxyl ions for the ionized impurities in the water.

Distillation: A purification process involving the phase change of water from liquid to vapor and back to liquid, leaving behind certain impurities.

Electrodialysis: A purification process that removes impurities from water using an electrical current to draw ionic contaminants through ion selective membranes (ion exchange resin in sheet form) and away from the purified water.

Endotoxin: The lipopolysaccharide fragments of bacterial cell walls, ranging in size from 15,000 to one million Daltons in size; considered pyrogenic if they have a fever inducing effect.

Exhaustion: The state in which an ion exchange resin is no longer capable of useful absorption: the depletion of the exchanger's supply of available ions. The exhaustion point is typically determined in terms of the reduction in quality of the effluent water as determined by a conductivity bridge which measures the resistance of the water to the flow of an electric current.

Exotoxin: A toxic substance secreted by a bacterium, often causing disease, such as tetanus or botulism.

Feedwater: The water brought to a filtering method before it is filtered; the water entering a purification system.

Hardness: The scale-forming and lather-inhibiting qualities which water possesses when it has high concentrations of calcium and magnesium ions. Temporary hardness, caused by the presence of magnesium or calcium bicarbonate, is so-called because it may be removed by boiling the water to convert the bicarbonates to the insoluble carbonates. Calcium sulfate, magnesium sulfate, and the chlorides of these two elements cause permanent hardness.

Hardness as calcium carbonate: The expression ascribed to the value obtained when the hardness-forming salts are calculated in terms of equivalent quantities of calcium carbonate; a convenient method of reducing all salts to a common basis for comparison.

Hydroxyl: The term used to describe the anion (0H-) which is responsiblefor the alkalinity of a solution.

Ion: Any nonaggregated particle of less than colloidal size possessing either a positive or a negative electric charge.

LAL: Limulus Amoebocyte Lysate, a test for pyrogen/endotoxin levels. LAL is an extract from the horseshoe crab which forms a gel in the presence of sufficient pyrogens.

Glossary

Monovalent: An ion in solution that has given up or gained only one electron, represented by one plus or minus sign in front of the ion's symbol. Sodium ion (Na^+), chloride ion (Cl^-), and ammonium ion (NH_4^+) are all monovalent ions.

Mixed Bed Ion Exchange: A combination of anionic and cationic exchange resins mixed together in one container.

Nuclear Grade Resin: The quality of deionizing resin material required for the nuclear energy industry; the highest quality grade of resin.

Polish: The process of removing the remaining contaminants from a preprocessed feedwater.

Polishing Mixed Bed Resin: A mixed bed of cation and anion exchange resins designed for use in high purity water systems.

Pyrogen: A thermostable component of gram-negative bacteria cell walls that may cause a fever when injected or infused.

Reject water: The water from a reverse osmosis membrane which contains a higher level of contaminants than the feedwater that is carried out the drain; the concentrate.

Reverse Osmosis: A process in which water is forced under a pressure sufficient to overcome osmotic pressure through a semipermeable membrane leaving behind a percentage of dissolved organic, dissolved ionic, and suspended impurities, typically 90-100%. Product water quality depends on feedwater quality.

Silt Density Index: SDI: also called the Fouling Index: a test used to determine the concentration of colloids in water; derived from the rate of plugging of a 0.45 micron filter run at 30 psi pressure.

Specific Resistance or Resistivity: The electrical resistance in ohms measured between opposite faces of a one centimeter cube of an aqueous solution at a specified temperature. Resistivity is usually corrected to 25 C and expressed as megohms-cm.

Total Organic Carbon: TOC; measures the degree of contamination by microorganisms and organic compounds.

Total Dissolved Solids: TDS; a semi-quantitative measure of the sum total of organic and inorganic solutes in water.

Turbidity: Refers to the degree of cloudiness of the water caused by the presence of suspended particulate or colloidal material. In a photometric method, turbidity acts as an analyte by reducing the transmission of light; measured in turbidity units.

Ultrafiltration: A process in which water flows tangentially across a semipermeable membrane having a highly asymmetric pore structure. The membrane is tight enough to retain contaminants and macromolecules at its surface while allowing water to pass through. Typical pore diameter may range from 0.1 to 0.002 micron.

Ultraviolet: (Photochemical) Oxidation: A process using extremely short wavelength light than can kill microorganisms (disinfection) or cleave organic molecules (photo oxidation) rendering them polarized or ionized and thus more easily filtered from the water.

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