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The Production of High-Purity Water in the Clinical Laboratory

ABSTRACT *As the sensitivity and variety of tests increase, the types and tolerable concentrations of impurities that may inhibit clinical tests become an increasing concern. Because water constitutes a high percentage of the buffers and reaction mixtures used in clinical assays, the use of high-purity water is critical for reliable, consistent, and cost-effective laboratory analysis. A wide range of contaminants exists in potable water supplies and must be removed with a water system containing the proper combination of specific purification technologies. Technologies such as activated carbon, reverse osmosis, ion exchange, and distillation have particular purification capabilities and must be selected based on their ability to consistently produce water with purity suitable for specific test methods. The National Committee for Clinical Laboratory Standards (NCCLS) provides guidelines for the production and use of purified water in the laboratory. The production of reagent water that meets the NCCLS guidelines requires proper measurement and monitoring of contaminant levels in both incoming source water and product reagent water. The introduction of new diagnostic tests requires the removal and monitoring of contaminants, such as nucleases and extraneous DNA, and creates new criteria for water purity and water system design. Current water purification technologies are reviewed, and a system designed to provide type I water is discussed.*

This is the third part of a 3-part continuing education series on water. Upon completion of this article, the reader will understand the proper selection and use of water purification technologies applied in the laboratory.

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The Importance of High-Purity Water

The modern clinical laboratory has experienced dramatic changes due to the introduction of new test methods and rapid, automated sample processing systems.[1] New test methods, as well as the emphasis on accuracy, quality control, and cost-effective practices, demand the use of pure chemical reagents. Because water constitutes a high percentage of most reagent solutions, high-purity water is a critical chemical reagent that should be selected to meet or exceed the purity required for the specific application. Typical uses include:

- Reagent reconstitution
- Dilutions
- Blank and standard solutions
- Rinse solutions
- Buffer preparation
- Culture media
- Clinical analyzer feed

By defining the specific water purity needed to perform laboratory tests, one may determine the type of water purification system that will effectively remove unwanted contaminants.

Understanding Contaminants in Source Water

Water is an effective "universal" solvent for polar substances. However, this useful solvent property produces some very complex water chemistry conditions that require rigorous purification steps to produce water for laboratory use. The contaminants found in natural waters can be classified as follows:

- Particles
- Dissolved inorganics
- Dissolved organics
- Dissolved gases
- Microorganisms

An accurate analysis of the levels of specific contaminants in source water is necessary before the installation of a water purification system (Table 1). The results from the source water analysis are used to determine the type and capacity of individual purification steps needed within the water system. Routine, periodic testing of incoming feed water is recommended because the concentration of contaminants may vary due to seasonal changes or maintenance performed by municipal water suppliers. The tests may be done at a reasonable cost using commercially available water chemistry test kits. Water chemistry tests should be performed with newly obtained samples, on site when possible. Storage or transport of water samples for testing often alters the sample due to changes in pH, bacterial content, or free chlorine concentration.

Water Purification Technologies

An effective laboratory water purification system must efficiently remove contamination that would interfere with specific tests or procedures. A water system is composed of a series of purification stages, each designed to remove a particular range of contaminants. Because no single technology alone will remove all types of contaminants effectively, a combination of purification technologies is needed. The following describes the major technologies used in the construction of a water purification system for the laboratory.

Activated carbon, also referred to as activated charcoal, is used to remove total and free chlorine and organic substances from water. Carbon from synthetic or natural sources, such as wood, coal, or coconut shells, is heat treated under special conditions to produce fine particles with high porosity and surface area. Oxidative free chlorine in water is reduced to chloride by the carbon, preventing the oxidation of reverse osmosis (RO) membranes and ion-exchange resins downstream in the water system. The removal of chlorine can also allow the growth of bacteria on carbon particles and components downstream within the water system. Carbon beds should be replaced periodically and may be flushed with hot water or acidic or caustic solutions to reduce bacterial accumulation. Organic substances are adsorbed to the surface of the carbon based on the pore size distribution and specific chemical composition of the carbon.

Depth filters effectively remove large particles found in drinking water. A depth filter may consist of a canister of fine sand or a polymer fiber or string-wound cartridge. Particles are retained by entrapment as they move through the tortuous path of the filter matrix. Depth filters protect expensive, fragile membranes downstream in the water system from damage.

Membrane filters consist of thin sheets of polymeric material designed to exclude particles larger than the rated pore size of the membrane. Microfiltration membranes capture particles on their surfaces and exhibit a more distinct particle removal profile than that of depth filters. Microfilters have pore sizes ranging from about 0.05 to 5 μm and can be selected to remove bacteria or other specific types of particles. Ultrafilters have pore sizes ranging from 1,000 to 200,000 daltons and may be selected to remove proteins, endotoxins, and high-molecular-weight organic compounds. Microfilters and ultrafilters are typically used to purify deionized water.

Table 1. Chemistry Tests Commonly Performed on Feed Water Sources

Measurement	Units	Typical Range in H_2O	Test Method
Conductivity	μS/cm	50-2,000	Meter with external probe
Particle fouling	Silt density units	4-30	Silt density level
Chlorine (total)	mg/L	0-3	DPD chemical test
Chlorine (free)	mg/L	0-3	DPD chemical test
Hardness	mg/L	0-200	Calcium chemical test
pH	pH units	5-8	pH electrode or chemical
Silica	mg/L	0-25	Molybdate chemical test
Iron	mg/L	0.0-0.5	Soluble iron chemical test
Bacteria	CFU/mL	5-2,000	Total count dip or plate

CFU, colony-forming units; DPD, N,N-diethyl-p-phenylenediamine indicator reagent.

Membrane filters with small pore sizes are subject to fouling and must be replaced frequently or operated in a tangential flow mode that continuously removes accumulating fouling materials from the membrane surface.

In reverse osmosis, water is forced via hydraulic pressure through a membrane that excludes ionized species and materials with molecular weights above 100 to 200 daltons. The pressure applied to the water must be significantly greater than the opposing osmotic pressure generated by the concentration gradient across the RO membrane.

RO is an effective method to remove 90% to 99% of particles, ions, organics, and microorganisms from potable source water in a single step. However, due to the small pore size of RO membranes, the product flow rate for laboratory-scale RO systems is limited to about 120 L/h. Therefore, RO systems typically require use of a storage reservoir to supply immediate demands for large volumes of water. The efficiency of an RO system is measured by the percentage of ions rejected by the RO membrane, with acceptable performance ranging from 90% to 99% ionic rejection. RO membranes require pretreatment with particle filtration and chlorine removal plus periodic maintenance to remove biofilms, mineral scale, and organic accumulation.

Ion exchange involves the removal of ions from water using a chemically treated polymer substrate called an ion-exchange resin. The ion-exchange resin consists of charged chemical functional groups attached to a polymer backbone. The charged groups attached to the resin are in equilibrium with an oppositely charged counter ion. When an ion in the bulk water phase has a greater affinity for the functional group of the resin than the bound counter ion, the ion in the bulk water phase chemically bonds to the resin and liberates the counter ion into solution.[2]

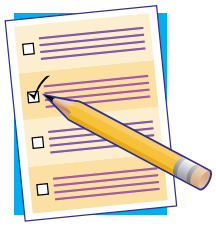
Two common applications for ion-exchange resins are water softening and water polishing. The resins used in water softening remove scale-forming cations, such as calcium and magnesium, in exchange for non-scale-forming sodium ions. Water polishing devices contain a balanced mixture of resins with either H^+ or OH^- as the bound counter ion. Ions in the bulk water phase chemically bond to the resin, which subsequently releases H^+ and OH^-, the components of pure water. Polishing resin systems are the most effective method to produce water with 18 megohm-cm electrical resisitivity. Electrical resistivity, the mathematical inverse of conductivity, is a practical measure of ionic purity for highly purified water. High resistivity values correspond to low concentrations of ions in a solution (Table 2). Water systems containing ion-exchange resins must be monitored with resistivity sensors to detect the exhaustion of the resin capacity and prevent the breakthrough of ionic contamination. Ion-exchange resins do not remove neutral species and can leach organic contaminants in small quantities. Silica is especially difficult to remove because it is weakly ionized in water at neutral pH. Stagnant water in cartridges or reservoirs containing resin can support bacterial growth. As a result, systems using ion exchange should be designed to periodically recirculate water to minimize extractable contaminants and bacterial growth, thus allowing delivery of high-purity water immediately upon use.

Distillation involves the heating of water to a volatile vapor phase to leave behind nonvolatile impurities and the subsequent cooling of the water vapor to liquid to remove it from highly volatile components. A single-stage still is composed of a water supply to a boiler followed by a device that guides the steam away from contaminating droplets and a condenser that liquifies the steam. Stills can effectively remove particles, ions, and a high percentage of dissolved gases.

Distillation is an excellent process for the removal of bacteria and endotoxins. It is common to process water through multiple distillation steps. Stills may yield a 100-fold to 10,000-fold purification factor dependent on the quality of the incoming feed water. High levels of hardness or silica in feed water can result in scaling of the interior

Table 2. Calculated Resistivity and Conductivity Values for Concentrations of Sodium Chloride in Water at 25°C

NaCl (µg/L)	Resistivity (MΩ-cm)	Conductivity (µS/cm)
0.0	18.18	0.055
1	17.60	0.057
5	15.20	0.066
10	13.10	0.076
20	10.20	0.098
50	6.15	0.16
100	3.70	0.27
300	1.43	0.70
500	0.88	1.13
1,000	0.45	2.21
5,000	0.093	10.80
20,000	0.023	42.70



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surfaces of the still and impaired heat-transfer properties. Also, it is difficult to remove materials that volatilize at temperatures near the boiling temperature of water.[3] Most laboratory-scale stills produce water at relatively low flow rates and require a storage reservoir to supply significant volumes of water on demand. Distilled product water has a maximum resistivity of approximately 1 to 5 megohm-cm mainly due to the exposure of final product water to the atmosphere and the glass construction of the still components.[3] Also, the high heat required for vaporization of water (8,080 BTU/gal) demands that large amounts of energy be consumed to continuously produce steam in the distillation process.

Two UV wavelengths, UV_{254} and UV_{185}, are useful for water purification.[4] UV_{254} effectively breaks bonds between carbon, nitrogen, and hydrogen atoms. As a result, UV_{254} destroys living microorganisms by disrupting their DNA. The destruction of various organisms is UV dosage-dependent. UV_{254} also exhibits good penetration in air and water, making it useful in disinfecting process water streams and storage reservoirs. UV_{185} carries more energy than the longer UV_{254} wavelength. UV_{185} not only breaks organic bonds, but also generates chemical species called free radicals. Free radicals are short-lived, highly reactive molecules or atoms that can rapidly oxidize organic and inorganic molecules. Free radicals ionize neutral organics, causing an increase in the conductivity of the water. The ionized organic molecules can then be removed using ion-exchange resins. The use of ion-exchange resins downstream of UV_{185} oxidation lamps allows the continuous production of ultrapure water with low organic content without the addition of chemical oxidants. The treatment of water with UV oxidation removes organic impurities and improves consistency and sensitivity in applications[5] such as:

- High-performance liquid chromatography (HPLC)
- Ion-exchange chromatography
- Solid-phase extraction
- UV spectroscopy

The successful production of high-purity water depends on the careful integration of water purification steps and system design and proper periodic maintenance (Table 3). Particle and carbon filters used for feed water pretreatment must be replaced before complete exhaustion to prevent fouling and chlorine breakthrough. Storage reservoirs should be designed to permit complete drainage of water with no stagnant volumes and be equipped with a venting device to remove particles and volatile substances from incoming air. Large distribution systems should be designed to permit complete recirculation of water with no stagnant areas of pipework. The final dispensing device of a water polishing system must be constructed of clean,

Table 3. The Effectiveness of Various Water Purification Technologies

	Contaminant								
Technology	Ions	Silicate	Bacteria	Organics	Particles	Gases	Pyrogens	Nucleases	Nucleic Acid
Depth filtration	P	P	P	P	G	P	P	P	P
Activated carbon	P	P	P	G	P	P	P	P	P
Reverse osmosis	G	G	F	E	E	P	F	P	P
Distillation	G	F	E	G	E	F	E	F	F
Ion exchange	E	E	P	F	P	F	F	F	F
UV 254-nm sanitization	P	P	G	P	P	P	P	P	P
UV 185-nm oxidation	P	F	G	E	P	P	P	F	F
Ultrafiltration	F	F	E	F	E	P	E	E	E
Microfiltration	P	P	E	P	E	P	P	P	P
Degasification	P	P	P	P	P	E	P	P	P

P, poor; F, fair; G, good; E, excellent

high-purity materials with minimal levels of extractable contaminants. The use of long pieces of tubing that retain stagnant water must be avoided, and filters used for bacteria removal should be replaced before bacteria levels increase from breakthrough or contamination from the environment.

Definition of Water Purity Types

The primary reference for the use of water in the clinical laboratory is the National Committee for Clinical Laboratory Standards (NCCLS) guideline, "Preparation and Testing of Reagent Water in the Clinical Laboratory," ed 3 (document C3-A3, October 1997). The NCCLS recommends that water meet specific levels of purity for ionic content (resistivity), bacterial content, pH, and silica concentration. Additionally, the NCCLS indicates that specific purification technologies be used to control particles and organic contaminants. The water purity is divided into ranges called "types" (Table 4). It is important to note that other organizations, such as the American Society for Testing and Materials (ASTM), may recommend water purity guidelines that differ from the NCCLS guideline because the ASTM guideline serves a different range of applications.

Type I water is considered ideal for procedures that are highly sensitive to contamination, such as enzymatic assays and tests involving nucleic acids. Because type I water must have greater than 10 megohm-cm resistivity, it must not be stored in tanks or carboys because ionic and organic contaminants will leach from the atmosphere and container materials. Although water may be at type I purity when newly dispensed into reagent bottles, the specifications for type I water cannot be obtained from commercial bottled water because storage introduces impurities.[6,7(pp9-10)] Type I water should be used immediately upon dispensing. The organic and particulate specifications for type I water require the use of activated carbon and a 0.22-µm filter within the system. The maximum tolerable concentrations of organics and particles are not specified and should be monitored by the user to confirm consistent and suitable purity.

Type II water is suitable for standard laboratory tests and procedures that are not affected by small amounts of ions, silicates, and bacteria. High-purity water that has equilibrated with atmospheric carbon dioxide reaches a resistivity of approximately 1 megaohm-cm and meets the ionic recommendation for Type II water. Type II water may be stored for short periods in a properly designed storage reservoir. In certain cases, a modified specification is used to conform to the requirements of the application (eg, type II water with a type I bacterial level).

Type III water is typically used for the washing and rinsing of glassware. It is critical, however, to ensure that no residual contamination that interferes with the test procedure is left on the glassware. The type and amount of residual contamination present on a surface are a function of the properties of the material as well as the concentration of contaminants in the water. For example, endotoxin has a high affinity for polypropylene and, therefore, should never be tested using polypropylene sample tubes.[8] In certain cases, a type I or type II water final rinse is necessary.

Water Purity Criteria and Testing

Silicates may be difficult to remove from water and can interfere with test results.[9(p8)] The performance of clinical assays in the presence of silica should be

Table 4. NCCLS Reagent Water Purity Recommendations for Clinical Laboratory Applications

	Type I	Type II	Type III
Resistivity minimum (megaohms-cm)	10	1	0.1
Microbial count (CFU/mL)	10	1,000	NR
Silicate maximum (mg/L)	0.05	0.1	1
pH	NR	NR	5.0-8.0
Particles	0.22-µm filter	NR	NR
Organics	Activated carbon, distillation, reverse osmosis	NR	NR

CFU, colony-forming units; NCCLS, National Committee for Clinical Laboratory Standards; NR, no recommendation.

determined and documented. Measurement of silica with the molybdate method yields accurate results to approximately 0.1 mg/L. The molybdate method will detect primarily dissolved ionized silica, but not colloidal silica. Atomic absorption spectroscopy or another method capable of measuring all forms of silica must be used to test for total silica.

Bacterial assays must be selected to provide adequate sensitivity to detect small numbers of organisms. When developing a protocol to obtain bacterial counts, a suitability study should be performed to determine the optimal sample volumes and conditions that yield accurate and precise results. A nutrient agar formulated for the recovery of stressed organisms (due to the low concentration of available nutrients) should be used to obtain optimal results from high-purity water. Although the calibrated loop spread method is commonly used to count bacteria, the NCCLS does not recommend this method for the analysis of bacteria levels in water due to the method's lack of sensitivity.

The pH measurement of high-purity water is challenging due to the absorption of carbon dioxide and the effect of very low ionic strength solutions on pH electrodes. Therefore, pH is a useful specification for type III water, but not for type I or type II water. In cases where the pH of high-purity water must be measured, a salt, such as potassium chloride, must be added to the water sample to increase the ionic strength without increasing the concentration of hydrogen ions. Water with a resistivity of 18.2 megohm-cm will have a pH of 7 based on the known concentration of hydrogen ions in pure water.

The recommendations for type I organic purity require that activated carbon be used in the purification scheme of the water system. No organic recommendation exists for type II or type III waters. Because the types and selectivity of activated carbon vary, the concentration of organics in the product water should be tested with consideration for the application. High concentrations of organics can modify surface tension and droplet formation or interfere with spectroscopic tests involving UV absorbance. Organic contamination increases background and alters column performance in HPLC.[5,6] Some organic substances increase the background of fluorescent assays or can inhibit cell growth.[9]

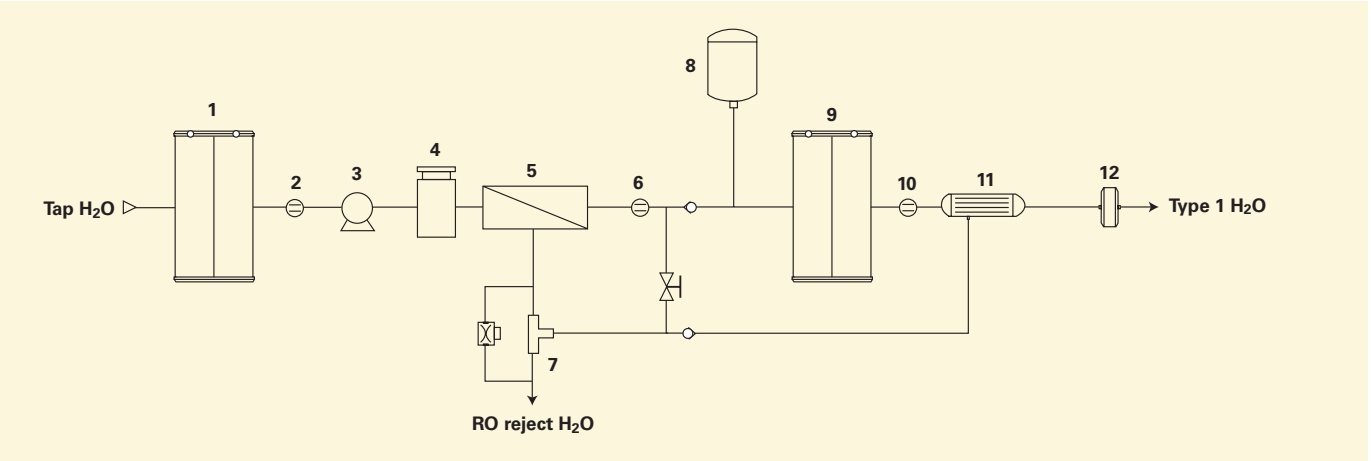
Particle removal for type I water requires the use of a 0.22-μm filter. No particle removal specifications exist for type II or type III water. A 0.22-μm filter device will remove bacteria but cannot guarantee complete sterility if used in a nonsterile environment.

The introduction of new assays and test methods is creating new criteria for water purity. However, some contaminants of concern are not listed in the NCCLS guideline and are classified under "special reagent water." It is the responsibility of the technician or investigator to establish suitable water purity levels for specific contaminants that affect a clinical assay.

Endotoxins can inhibit mammalian cell culture and should be monitored using the *Limulus* amebocyte lysate (LAL) test available commercially.[8] LAL is a reagent that reacts with endotoxins (also referred to as pyrogens) and is used to monitor endotoxin levels in buffers and water.

Nucleases destroy nucleic acids used in DNA amplification and screening assays. Ribonucleases are commonly inactivated by the addition of diethyl pyrocarbonate (DEPC). It is suggested that DEPC covalently modifies and inactivates ribonucleases.[11] However, the addition of DEPC adds ionic and organic contamination to the water and may inhibit some useful reactions of the nucleic acids of interest. Nucleases can be removed by using ion-exchange resins followed by ultrafiltration, thus eliminating the need for DEPC addition.[12] Bacteria can contaminate water with nucleic acids that interfere with the resolution or amplification of nucleic acids of interest.[13] It is critical, therefore, to adhere to the maintenance instructions of the water system to minimize microbial contamination.

Dissolved gases exist in either ionized or nonionized forms in water. Oxygen and nitrogen do not ionize in water under normal conditions, whereas carbon dioxide, ammonia, and chlorine partially ionize via the formation of weak acids or bases. Because most gases undergo a decrease in solubility with a respective increase in temperature, gases will evolve from most aqueous solutions that undergo a dramatic increase in temperature. Water entering a water purification system at 10°C will release gases when heated to 37°C in a test procedure, for example. If the heating occurs inside of a clinical analyzer, the small gas bubbles generated can block fluid lines or interfere with optical sensors. Gases can be removed efficiently using a hollow-fiber degassing cartridge. Water containing a high concentration of dissolved gas passes through fine capillaries made of a hydrophobic polymer. When a vacuum is applied on the exterior of the fibers, gases are removed from the water, which is



Water purification system designed to automatically supply type I (National Committee for Clinical Laboratory Standards), degassed water to an automated clinical analyzer system. 1, Pretreatment cartridge (particle/carbon filtration); 2, conductivity sensor; 3, pump; 4, sanitization port; 5, reverse osmosis (RO) membrane; 6, conductivity sensor; 7, vacuum eductor; 8, RO water bladder tank; 9, ion-exchange resin cartridge; 10, resistivity sensor; 11, degassing module; 12, 0.22-µm membrane filter.

processed and sent to the clinical analyzer. If the temperature of the degassed water is increased within the analyzer's fluid transfer lines or reaction cuvettes, no gas bubbles are formed.

The automation of new clinical assays will demand the use of systems that will deliver high-purity water automatically. For example, an integrated on-line water purification system provides continuous operation, type I purity, and consistent water quality using a combination of activated carbon, depth filtration, reverse osmosis, ion exchange, degassing, and membrane filtration (Fig). In the future, water systems should incorporate sensors capable of monitoring other critical substances in water, such as organics and DNA, as assays become sensitive to such contaminants. Additionally, the guidelines for water purity must include specifications for new impurities that must be controlled for the use of polymerase chain reaction, complex nucleic acid hybridization assays, and ultrasensitive detection systems. Clinicians will need to consider the impact of new waterborne contaminants and develop comprehensive protocols for water system maintenance and monitoring.1

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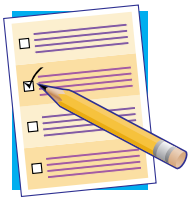

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7	☐	☐	☐	☐	☐
8	☐	☐	☐	☐	☐
9	☐	☐	☐	☐	☐
10	☐	☐	☐	☐	☐

Multiple-Choice Questions

What factors in diffusion-controlled membrane processes affect the rejection of contaminants by reverse osmosis and the nanofiltration process?
A. Pressure
B. Charge
C. Size
D. Solubility
E. All of the above

2. The "CTs" used for development of disinfection regulations in potable water are:
A. a simplified version of the "Chick-Watson" law.
B. the product of disinfectant concentration and time.
C. specific for each organism regulated by USEPA.
D. specific for each disinfectant
E. all of the above.

3. Select the membrane process that would not be capable of removing protozoan cysts:
A. Microfiltration.
B. Nanofiltration.
C. Ultrafiltration.
D. Electrodialysis reversal.
E. Reverse osmosis.

4. The purification media most effective for the removal of chlorine from tap water is
A. ultrafiltration.
B. ion exchange resin.
C. granutar activated carbon.
D. degassing.
E. sand filter.

5. The NCCLS recommendation for silicate in Type II water is
A. 0.1 g/L.
B. 18.2 mg/L.
C. 10 mg/L.
D. 0.1 mg/L.

6. When performing chemical analysis of tap water, the samples should be
A. stored at 4°C.
B. analyzed on site as soon as possible.
C. diluted to eliminate interferences.
D. degassed.
E. kept out of direct sunlight.

7. Coagulants are designed to do which of the following?
A. Sterilize untreated water.
B. Keep small particles suspended in liquids.
C. Make solid particles stick to each other.
D. Decrease the amount of solids removed from water samples.
E. Counteract the effect of gravity on waterborne solids.

8. Which of the following removes microorganisms by sieving?
A. Coagulation
B. Filtration
C. Suspension
D. Flocculation
E. Chlorination

9. Which of the following chemicals is most often used as the primary disinfectant during drinking water treatment?
A. Hypochlorus acid (chlorine)
B. Chloramines
C. Aluminum sulfate
D. Ferric chloride

10. Which choice has the microorganism(s) most resistant to disinfection?
A. *Giardia* cysts
B. *Cryptosporidium* oocysts
C. Poliovirus
D. *Escherichia coli*
E. A & B

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