*The formulae* $\frac{\partial \rho U_i}{\partial t}+\frac{\partial}{\partial x_j}(\rho U_i U_j)=-\frac{\partial P}{\partial x_i}+\frac{\partial}{\partial x_j}\left(\mu\frac{\partial U_i}{\partial x_j}\right)+g_i(\rho-\rho_0)$ $\frac{\partial \rho U_i k}{\partial x_i}=\frac{\partial}{\partial x_i}\left(\left(\mu+\frac{\mu_T}{\sigma_k}\right)\frac{\partial k}{\partial x_i}\right)+P+G-\rho\varepsilon$ *for building* $\frac{\partial \rho U_i \varepsilon}{\partial x_i}=\frac{\partial}{\partial x_i}\left(\left(\mu+\frac{\mu_T}{\sigma_\varepsilon}\right)\frac{\partial \varepsilon}{\partial x_i}\right)+(C_1-C_{1RNG})\frac{\varepsilon}{k}(P+C_3G)-C_2\rho\frac{\varepsilon^2}{k}$ $\frac{\partial}{\partial x_i}(\rho\overline{U_i H})=\frac{\partial}{\partial x_i}\left(\lambda\frac{\partial \overline{T}}{\partial x_i}-\rho\overline{u_i' h'}\right)$

$-\rho\overline{u_i' u_j'}=\mu_T\left(\frac{\partial \overline{U}_i}{\partial x_j}+\frac{\partial \overline{U}_j}{\partial x_i}\right)-\frac{2}{3}\rho k\partial$ *state of the art* $\frac{\partial}{\partial x_j}(\rho\overline{U}_i\overline{U}_j)=-\frac{\partial P}{\partial x_i}+\frac{\partial}{\partial x_j}\left(\mu\frac{\partial \overline{U}_i}{\partial x_j}-\rho\overline{u_i' u_j'}\right)+g_i(\rho-\rho_0)$ $\frac{\partial \rho U_i \varepsilon}{\partial x_i}=\frac{\partial}{\partial x_i}\left(\left(\mu+\frac{\mu_T}{\sigma_\varepsilon}\right)\frac{\partial \varepsilon}{\partial x_i}\right)+C_1\frac{\varepsilon}{k}(P+C_3G)-C_2\rho\frac{\varepsilon^2}{k}$ *biomedical research facilities.*

NIH National Institutes of Health
*Turning Discovery Into Health*

# Laboratory Water

## *Its Importance and Application*

***March, 2013***

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## Overview

We often take water quality for granted in daily life and in our work. If we work in a laboratory, we may be very conscious of the need for reagent grade water (RGW) for our laboratory experiments and animal water in order to reduce the risk of scientific variability or to prevent bacterial disease respectively, yet we fail to consider the quality of the water we use in our equipment. We also might not be aware of the different grades of water that are available, the appropriate water grade applications or the cost to obtain the desired water grade. Inattention to water quality in the lab can result in compromised experimental results, contaminated reagents or damaged equipment. Biomedical research, medical, and design professionals should become familiar with and apply the water grade most applicable to their needs. The Division of Technical Resources (DTR), Office of Research Facilities (ORF) has written several articles about water quality that may be of interest.

Policies and Guidelines pages DRM_News_to_Use

Water is known as the universal solvent because more substances (not all substances) dissolve in water to varying degrees than in any other solvent. This is due to the unique polarity and hydrogen bonds of the water molecule. The same unique molecular properties of water account for its ability to react with neutral organic molecules and establish hydrogen bonding with other molecules. For this reason, water quality is crucial in the laboratory because wherever water is required, its reactivity must be taken into account. Water is easily contaminated by chemical solids, gases, vapors and ions that leach from conduit lines and containers. These may include sodium and silica from glass, plasticizers and ions from piping, microbial species and their endotoxins, as well as particulate contaminants. (Millipore, n.d.) Soluble organic contaminants can even be introduced from deionizer resins used in the treatment process, especially if inadequate resins are selected or resins were previously contaminated.

To avoid the risk of contamination and ensure appropriate economies, centralized laboratory water systems should be designed to meet the 'process' or 'product' water (PW) grade necessary for the most common applications, and to provide quality feedwater suitable for final polishing to serve ultrapure applications, such as typically required for various analytical applications.

## What are common water contaminants?

Natural or 'tap' water contains many substances that if left untreated may react or catalyze reactions in undesired ways. Cations such as sodium, calcium, magnesium or iron; anions such as bicarbonate, chloride and sulfate; and inorganic ions, are found in tap water. Dissolved biological organic molecules, gases such as nitrogen, oxygen and carbon dioxide as well as hard and soft particulates and colloids may be introduced into the tap water from any number of sources. Volatile organics such as lower hydrocarbon trace pollutants from farm water runoff and industrial pollution, and contaminants introduced as a consequence of treatment processes such as trihalomethanes (THMs) as a byproduct of chlorination may be present. Although many bacteria, viruses, and cysts such as giardia and cryptosporidium found in tap water are killed or inactivated by the local chlorination process, microbial by-products and cellular fragments such as pyrogens, nucleases, alkaline phosphatase and endotoxins

will not necessarily be removed and can proliferate in biofilms. A number of contaminants may be present that are not regulated or tested in the course of municipal potable water treatment and distribution, yet are detrimental to experimental processes that require precision, high sensitivity and resolution and instrumental operation and longevity.

## How do we measure contaminants in water?

Resistivity and conductivity are concepts to be familiar with when it comes to water purity. Resistivity is the tendency of water without ions to resist conducting electricity. The unit of measure is megohmcentimeter (MΩ-cm), and varies with temperature. The theoretical maximum is 18.2 to 18.3 MΩ-cm at 25°C. The higher the ionic content, the lower the resistivity and conversely, the lower the ionic content, the higher the resistivity. In ultrapure water systems this value is determined using an in-line meter (ThermoScientific, 2009). Resistivity measurements taken upstream in a system prior to subsequent treatments such as UV, fine filtration, or even exposure to air, while important to monitoring certain treatment processes, may not be completely indicative of the final product water's ionic content.

Conductivity is the tendency of water that contains ions to conduct electricity. The unit of measure is the Siemen(S), microsiemens/centimeter (µS/cm) or micromho/cm. The measurement is used to measure feed water and lower qualities of treated water. The more ions present in the water, the higher the conductivity. This is measured by a conductivity meter (ThermoScientific, 2009), and for accuracy must be taken on-line. Conductivity increases with temperature so values are reported as compensated at 25 °C whereas resistivity is the inverse of conductivity and is expressed in 18.2 MΩ-cm@ 25 °C (Riley, 2012).

Measurement of ionic contamination is not in itself indicative of "pure" water. A number of contaminants must also be considered, including microbial and organic factors. An example to illustrate this point is the dissolution of 1,000 ppb of sucrose in theoretically pure water, while still achieving resistivity approaching 18.2 MΩ-cm at 25°C (LCGC North America, 2005). Further, certain ionic contaminants may lower resistivity, yet the result may not be indicative of poor water quality. For example, as little as 10 to 15 ppb of CO2 from clean air could cause the resistivity of 18.2 MΩ-cm water to drop to as little as 10 MΩ-cm (at 25°C). Limitations in accuracy of common instrumentation (even on-line type) as water approaches the theoretical levels of purity are often beyond that present in many laboratory water systems.

Total Organic Carbon (TOC) is a measure of the organic contaminants found in water. The unit of measure is parts per million (ppm) or parts per billion (ppb). High levels of TOC are indicative of organic contaminants, many of which may also serve as nutrients for microorganisms and be indicative of other contaminants. While elevated TOC readings do not identify the specific contaminant, a TOC reading when used along with conductivity and microbial parameters is an excellent qualitative indicator. Feedwater system TOC levels in the range of 200 ppb (and certainly below 500 ppb) could be considered practically reasonable feed water quality for central systems subject to final polishing. The best high

purity water (after polishing) should be in the 1-5 ppb range, and such low TOC levels can be critical for some applications (such as HPLC). High-performance liquid chromatography (HPLC) {sometimes referred to as high-pressure liquid chromatography}, is a chromatographic technique used to separate a mixture of compounds in analytical chemistry and biochemistry with the purpose of identifying, quantifying or purifying the individual components of the mixture. Typically when measuring TOC levels required of centralized feedwater systems, properly conducted off-line measurement at regular intervals (such as quarterly) is acceptable; however where highly sensitive TOC restrictions are required (such as <50ppb), on-line measurement should be performed. There are practical limits to the accuracy of reasonably economic TOC instrumentation, and if a particular contaminant is of concern, more direct testing for that contaminant may be required.

As potable water is further treated to achieve high purity for scientific applications, residual disinfectants are removed leaving distribution systems vulnerable to microbial colonization and biofilms. If left unchecked, the microbial qualities can rapidly deteriorate below potable water standards and a variety of contaminants most notably gram negative bacteria, fungi, algae, endotoxins and cellular waste products can reduce water quality, interfere with treatment processes, and reduce removal efficiency.

Plate count methods are often utilized to provide some measurement of water quality, however the limitations of such methods must be considered. Plate counts often significantly underestimate the quantity of viable organisms (partially due to the presence of biofilms) and do not account for microbial byproducts, inactive (non-replicating) microbes, or indicate presence of associated cellular material. Further, such tests when applied to single samples may not in themselves provide adequate representation of system microbial quality as variations can occur widely dependent upon system location, sample volume, and sloughing of biofilms. Much of the microbial contamination in purified systems may not actually be free-floating bacteria and for these reasons and to help achieve rapid results, the use of more accurate techniques such as epifluorescence microscopy coupled with endotoxin testing should be considered.

Endotoxin testing can provide a good indicator of gram negative bacteria and microbial byproducts, as well as many fungi and algae. The limulus amebocyte lysate (LAL) test is a quick and effective method of endotoxin testing, and is recommended for sensitive applications. Water samples that test positive with a glucan-sensitive endotoxin assay should be evaluated with a glucan-insensitive or glucan specific assay. It is always important to rule out cross-reactions and interferences by performing standard additions of known concentrations of endotoxin and/or a (1,3)-beta-D-glucan, depending on which entity is of interest. (CLSI C3-A4). For less sensitive applications and routine testing of feedwater systems, gel-clot may be considered. Endotoxin levels are measured in Eu/ml (endotoxin units per milliliter), and levels <25 and preferably below 2 to 5 Eu/ml can be considered representative of that found in good quality potable water. Significantly lower endotoxin levels should be present in ultrapure waters used for certain analytical applications (for example as required for cell culture and biopharmaceuticals production), and many polishers have capability to achieve endotoxin levels of

0.004 EU/ml, some to as low as 0.001 EU/ml. Most laboratory applications do not require sterile water, and where such is required it is typically produced or procured specifically for that purpose.

The control of ionic, organic, and microbial contaminants through measurement of conductivity, TOC, and microbial/endotoxin provides significant assurance of water quality. Where specific contaminants may be of concern however, it is important to confirm the process adequately addresses a particular need or contaminant.

## What does Lab Water Grade Mean and why does it matter?

Reagent grade water (RGW) is water that is suitable for use in a specified procedure such that it does not interfere with the specificity, accuracy, and precision of the procedure. Process definitions alone (e.g. "RO", "DI", "Distilled") do not in themselves adequately define required water quality. The quantitative parameters associated with attempts to standardize description of water quality vary widely amongst standards organizations and have been subject to on-going changes. Many of the parameters have qualifiers and ultimately defer to requirements as determined appropriate and validated by the end user. This makes sense in that the wide variety of applications can have substantial variation to tolerances with regards to the composition and quantity of contaminants. It is therefore not uncommon that water quality parameters be supplemented with identification of the intended applications, specific contaminant concerns, (and sometimes even purification process identification) to ensure intended results will be achieved. When communicating water quality and tolerance for contaminants, the question invariably becomes: "How pure is pure"?

Water specifications have been described by ASTM (American Society for Testing and Materials) D1193, ASTM D5196, ISO (International Organization for Standardization) 3696 and CLSI® (Clinical and Laboratory Standards Institute (formerly NCCLS) C3-A4. Historically waters of the highest purities have often been described as "Type I" to designate ultrapure waters, and Type II or Type III to designate lower grades. The ASTM standards specifies processes that should be used for the production of purified waters, however each process is qualified with allowances for other processes that are deemed to produce acceptable results.

The ISO utilizes the term "Grade" in lieu of type, with significant differences of criteria. The scope of the ISO standard is limited to laboratory reagent water for analysis of inorganic chemicals. The American Chemical Society (ACS) also has a standard for water used for reagents and contains specific limitations for various inorganic contaminants. The levels presently specified are unlikely to be sufficient for many biomedical lab water applications (for example, the resistivity parameter is 2 MΩ cm at 25°C).

CLSI does not utilize the terminology "Type I", "Type II" etc. typical of ASTM D1193 or the older NCCLS standard, but instead utilizes the designations CLRW (Clinical Lab Reagent Water) and other water designations such as SRW (Special Reagent Water) and Instrument Feed Water; neither of which are specifically defined and are intended to be determined on an local or application basis. In attempting to

perform a correlation between these varying parameters, the reader might consider applications of SRW to what is often thought of as Type I or Ultrapure waters, though CLRW could also in some cases fit such a description. It is important to recognize that ultimately water qualities and production methods are over-ridden in each of the standards by application specific requirements, and therefore all the standards are more effective when viewed as a guide. Nevertheless, the use of the terminology "Type I", "Type II" and "Type III" have familiarity and are useful in communications. Tables 1, 2, 3, and 4 help illustrate some of the variations between these standards.

**Table 1, NCCLS**
National Committee for Clinical Laboratory Standards
Reagent Grade Water Specifications

| Parameter | Type I | Type II | Type III |
|---|---|---|---|
| Bacteria, max. (CFU/ml) | 10 | 1000 | NS |
| pH, units | NS | NS | 5 - 8 |
| Resistivity, min. (megohm) | 10 | 1.0 | 0.1 |
| Silica, max. (mg/l) | 0.05 | 0.1 | 1.0 |
| Particles | 0.22 micron filtration | NS | NS |
| Organics | carbon filtration | NS | NS |

**Table 2,**
**Clinical Lab Standard Institute (formerly NCCLS), C3-A4 Reagent Laboratory Water**

| Parameter | CLRW | SRW | Instrument Feed |
|---|---|---|---|
| Microbial, max. (CFU/ml), plate count | 10 | Application Defined | NS |
| pH Units | NS | Application Defined | NS |
| Resistivity, min. (megohm) | 10 | Application Defined | NS |
| Silica | NS | Application Defined | NS |
| Particles and Colloids | 0.22 micron filter | Application Defined | NS |
| Organics (TOC), (ppb) | 500 | Application Defined | NS |
| Endotoxin | Application Defined | Application Defined | NS |

The ASTM establishes specifications for Types I, II, III, and IV reagent grade water (D1193-06-2011) as shown in Table 3. The water quality is further classified as Type A, Type B, or Type C depending on the applicable bacteriological and endotoxin quality. The ASTM also publishes standard 5196, Standard Guide for Bio-Applications Grade Water, with yet another set of parameters for water quality (Table 4).

**Table 3, American Society for Testing and Materials ASTM D1193-06 (2011)**
**Reagent Grade Water Specifications**

| Parameter | Type I | Type II | Type III | Type IV |
|---|---|---|---|---|
| Resistivity, min. MΩ-cm (25°C) | 18.0 | 1.0 | 4.0 | 0.2 |
| pH, units (25°C) | NA | NA | NA | 5 to 8 |
| TOC, max. (ug/l) | 50 | 50 | 200 | NS |
| Sodium, max. (ug/l) | 1 | 5 | 10 | 50 |
| Chloride, max (ug/l) | 1 | 5 | 10 | 50 |
| Total Silica, max. (ug/l) | 3 | 3 | 500 | NA |
| | Type A | | Type B | Type C |
| Bacteria, max. (CFU/100 ml) | 1 | | 10 | 1000 |
| Endotoxin (EU/ml) | <0.03 | | 0.25 | NA |

**TABLE 4, AMERICAN SOCIETY FOR TESTING AND MATERIALS, ASTM D5196-06 (2006)**
**Standard Guide for Bio-Applications Grade Water**

| Parameter | Bio-applications Water |
|---|---|
| Resistivity, min. MΩ-cm (25°C) | 18 |
| pH, units (25° | NS |
| TOC, max. (ug/l) | 20 |
| Sodium, max. (ug/l) | NS |
| Chloride, max. (ug/l) | NS |
| Total Silica, max. (ug/l) | NS |
| Microbial, max, CFU/ml | 100/100 |
| Endotoxin, max EU/ml | 0.01 (or as required) |
| Nucleases and Proteases | (as required) |

Additional water quality regulations exist for specific biopharmaceutical applications, such as water for injection (WFI), sterile waters for injection and USP purified water, as addressed in the US Pharmacopeia monograph. The USP contains design guidance for consideration in purified water systems and recognizes microbial concerns. USP purified water (which includes conductivity, TOC, and microbial parameters) may provide reasonable water quality for feedwater systems; however USP waters (including water for injection) would not meet

the requirements for many reagent lab water end use applications without additional treatment.

## Water Treatment Methodologies

The following methodologies are used for water treatment. The pros and cons for each method indicate the need for use of several methodologies to achieve the desired water purity.

### Distillation

Perhaps the best known method of water purification is distillation in which water is heated to the boiling point. Since distillation is a slow process, the water must be stored until use. During storage, contamination may occur whenever the container is breached or by leaching of minerals or compounds from the container into the distillate. Centrally applied distillation is often energy, time and labor intensive, expensive to maintain and not environmentally friendly. Poor still design or operation can readily result in inadequate performance, inconsistent water qualities, and pass through of organics or reintroduction of extracted contaminants. Distillation is however a versatile technology, capable of removing a wide range of contaminants and can be successfully applied locally for many applications.

### Filtration Methods

Filtration technologies (in descending order of particle capture efficiency) include reverse osmosis, nanofiltration, ultrafiltration, microfiltration and particle filtration. Filtration is used to pretreat water before it is further processed. Filters are designed to remove particles above the rated pore size of the filter in accordance with the filters efficiency or Beta Ratio at a given flux (or rate of flow) and are most often used at various points in the system to remove bacteria or other particles. Depending upon location, the rated pore size might be as high as 10 or 25 microns in pretreatment stages to as fine as 0.45µm to 0.2µm absolute at final treatment stages and points of dispense. Filtration is both efficient and easily changed out, but can become clogged and spread contamination if not routinely serviced. Filters cannot remove dissolved material.

Ultrafiltration (UF) methods are capable of removing bacterial endotoxins and nucleases which can affect tissue and cell culture procedures and media preparation. UF cannot remove dissolved material.

Reverse osmosis (RO) product water, possibly the most versatile water purification process, depends on the purity of the feed water and the effectiveness of the filter membrane. RO membranes are able to reject bacteria, pyrogens, inorganic and some organic solids but dissolved gases are not as effectively removed. The RO process is slow so a storage tank is required to collect and distribute the PW. RO requires pretreatment of feed water to avoid damage to the membrane by chlorine, mineral deposits, colloid build up and piercing by hard

particles. RO is an excellent primary treatment process, which when coupled with appropriate pretreatment including sorption (such as activated carbon) and fitted with UV oxidation inclusive of system designs with appropriate microbial controls, can provide a versatile water for many applications, and an economical baseline for final point-of-use polishing systems.

**Deionization**

Deionization (DI), demineralization or ion exchange removes ions from feed water using synthetic resins. Deionization (including electrodeionization) is the only technology which consistently produces the resistivity requirement for Type 1/Ultrapure RGW. Cation and anion resins are often mixed together for laboratory use to provide complete deionization. DI columns have a finite ion binding capacity during a cartridge life time; they do not remove particles, pyrogens or bacteria; and have very limited effectiveness with many organics. The quality and purity of ion-exchange resins can be a significant concern, especially with off-site regeneration. Ion exchange beds can be a haven for microbial growth and release of particulates. In order to consistently deliver appropriate water quality, deionizers must include appropriate pre-treatment, monitoring, and maintenance. Water that has been deionized is often referred to as “hungry water”, easily contaminated and capable of inducing corrosion in many materials.

**Electrodeionization**

Electrodeionization (EDI) combines electro-dialysis and ion exchange technology to remove ions from purified feed water. It is both effective and efficient because the EDI module is continuously recharged through the electric current from the unit. Although there are obvious advantages to a system that continuously and automatically regenerates itself, the feed water must be of high quality. EDI does not remove organics, particles, pyrogens or bacteria, though may be less prone to microbial contamination as compared to ion exchange resin beds.

**Adsorption**

Adsorption is used to remove chlorine, and chloramines from feed water via a high surface area activated carbon, and if properly sized and appropriately selected can also effectively reduce organics, measured as Total Organic Carbon (TOC’s). Adsorption may be combined with other methods to achieve maximum resistivity and low TOC. Adsorption techniques alone do not remove ions and particulates.

**Ultraviolet Oxidation**

Photochemical oxidation with ultraviolet light (UV) can eliminate trace organics at 185nm and inactivate microorganisms at 254nm.The oxidation of trace organics results in pure water with low TOC levels but does not remove ions, colloids, or particulates. The oxidation of organics

often results in decreased water resistivity due to the ionized contaminants (such as elevated carbon dioxide), which may require further downstream treatment to resolve.

## How Central Purified Water is Produced and Design Options

Water purification is not an exact science, can be highly dependent upon local (and even site-specific) variations in make-up water quality, as well as end-use requirements. Some techniques are better than others at removing certain contaminants and it is often necessary to employ several techniques in an integrated system to achieve the required water purity level.

Potable water that meets Federal Standards is the starting component to produce varying grades of high purity water. Centralized pre-treatment components (such as activated carbon to remove chlorine and reduce organics), water softeners (to reduce hardness and prevent RO membrane fouling), RO (to reduce ionic and organic contaminants and particles) and preliminary filtration (to prevent RO membrane fouling) are added to the system. 185nm UV may be incorporate to reduce TOC's. In-line monitoring devices described further on may also be added. This treated water is stored in a clean, non-contaminating opaque tank with 0.2µ air filtration and sometimes ozonated, heated, or routinely disinfected to limit introduction of microorganisms.

A properly designed circulation loop to avoid stagnant sections (dead legs) with ultraviolet light at 254nm and submicron filtration should be used for microbial control within the distribution system. The entire system should employ high quality materials to prevent contaminants leaching into the water. The process to this point provides what is sometimes referred to as "Type 3" Water of various microbial grades. Such water can be used at sink taps for non-critical purposes (such as rinsing), and at equipment connections (autoclaves, glasswashers, incubators, heating baths etc.) and to feed point of use "Polishers". This process will allow for efficient generation of high quality waters (such as "Type 1"ultrapure) to meet the individual research needs. It is important to shield piping from light (algae), protect systems from backflow, and perform periodic sanitizations, water sampling, equipment maintenance and fitness for use validations. System maintenance is critical to avoid continual contamination from particulates, bacteria, chemical leaching and absorption of atmospheric gases and vapors, or other breakthrough of contaminants. Types II and III water may be stored and distributed as needed but the system should include a measuring device to protect the chemical and microbial water quality. The system must be sanitized frequently, at least twice a year and sometimes quarterly. 90° C is the acceptable sanitizing temperature. Alternative sanitization methods include hydrogen peroxide/peracetic acid solutions, as well as ozonated water, however in all cases system materials must be compatible with the cleaning method. It is far easier to control biofilms through appropriate design and maintenance than to effectively clean a heavily contaminated system.

Higher purity systems typically use similar arrangements for a baseline, but may also incorporate second pass reverse osmosis, distillation, degassifiers, and/or deionizers in the treatment train, as well as more

rigorous controls, monitoring, and maintenance protocol. Local water conditions may also require variations in the design or arrangement of the pre-treatment train.

An example of a typical polisher capable of producing Type I ultrapure water when fed from an appropriate pre-treated water supply typically consists of an internal circulation pump, a granular activated carbon filter to reduce organics; a primary mixed-bed deionizer to achieve a maximum of 20 uS/cm conductivity, and a polishing mixed-bed deionizer to provide final water quality. The system may also include an in-line conductivity sensor and monitor, water dispensing faucet with a 0.2 micron post-filter capsule, and a recirculation pump. Optional components could include a 185 nm ultraviolet system installed before the polishing deionizer to provide for trace organics removal (dependant on manufacturer TOC less than 3 ppb), an ultra-filter membrane for endotoxin removal and a 254 nm ultraviolet unit for bacteria control, a UV intensity monitor for monitoring UV efficacy, a TOC on-line analyzer, and remote connections for batch validations to a computer or printer. Where units are placed on a shelf, units with capability for a remote dispense gun, remote mount wall or bench top outlet, and accessible user interface are often selected.

Polishers and point of use treatment equipment must be routinely maintained and sanitized, typically with peroxide or bleach solutions, often quarterly or semi-annually. Final filters may require frequent replacement or (where compatible) autoclaving. Polisher systems that are fed from a quality feedwater source and that incorporate these optional features can produce water that is essentially pure of almost all impurities, and often suitable for the most stringent applications (such as HPLC). There can be a wide variation in the treatment process, features, and materials of construction amongst polisher manufacturers, consequently equipment should be carefully evaluated.

For applications where water source is direct from tap water, a pre-treatment localized RO or distillation step is typically required. It is important to note that the throughput of polishing mixed-bed deionizers can be increased by as much as 6 to 8 times by using RO for primary water treatment, and for some ultra pure applications, required purity may not be met without adequate pretreatment.

It is important to prevent degradation of resistivity if the Type III water is stored. To accomplish this, a polishing deionization system might be installed after the storage tank or (in cases of ultrapure water) a nitrogen blanketing system on the storage tank may be used to prevent the absorption of carbon dioxide into the stored water. This is an important point to recognize in the use of ultrapure water, i.e. water once dispensed can become rapidly contaminated from contact with air or storage containers.

Other design considerations include sizing of equipment items to meet capacity and peak usage demands, utility requirements, space availability and access, instrumentation and control requirements, maintenance requirements, and future needs. System validation, quality control, and wastewater treatment should also be considered when designing the pure water system. (Riley, 2012). Purified water systems must be appropriately designed to maintain proper flow direction and system pressures under all demand conditions, to minimize risk of contamination and disruptions, and to avoid interconnections with other specialized systems (such as animal drinking water, aquatics, USP, or WFI).

Direct or reverse return distribution approaches should be utilized, and appropriate one-way valves (backflow preventers) or break tanks should be provided at potential sources of contamination (such as lab equipment and serrated tip faucets). It is especially important to maintain strict controls over materials, equipment connections, piping arrangements, SOP's and maintenance to maintain delivered water quality.

It is more expensive to produce Type 1 water than Type 2 or Type 3 water but often a lab will chose a higher grade of water than they need because they feel there is less chance of compromising their experiments. It is to the labs benefit both economically and technically to determine the grade of water required most frequently before selecting the water treatment or point of use system. Where and when a higher grade water is required, a smaller point of use system can be installed for those specific applications. For example, there is no need to use the most expensive grade of water to wash glassware. On the other hand, it is critical to use the specified water quality for sophisticated analytical processes where contaminant interference will affect the results, and sometimes the economies and spatial requirements associated with multiple systems may drive the approach. Ultrapure, high resistivity water is inherently aggressive, and can result in corrosion of laboratory equipment if improperly applied.

**The Best Solution**

For most large scale laboratory applications, the use of a well-prepared, centrally distributed purified feed water approach with point of use polishers should be implemented. Generally, the most basic and common application that requires the bulk of the water usage in a lab should be the default criteria in establishing the system design (typically feedwater to polishers). Point of use final polishers can then be selected by individual researches to meet the scientific needs of their program for ultrapure water, along with consideration of required degree of flexibility for analytical processes and usage volume within their labs. The unpolished water will often be suitable for a myriad of supportive applications, and can provide flexibility of a quality water source for application of point of use polishing wherever necessary.

Unlike the semi-conductor industry, it is not often cost effective to produce and maintain ultrapure waters for central distribution in laboratories. When central ultrapure (Type I) systems are applied, it is not uncommon to find that the water is actually too pure or uneconomical for a number of routine applications, yet "not pure enough" or of questionable certainty for other highly critical applications due to a number of potential variables, maintenance and monitoring requirements and potential for upstream contamination. Nonetheless, a high quality feedwater is paramount to serving the needs of many applications, and to achieving expected performance and operating economies from polishers.

High quality central feedwater (typically product water of a treatment process incorporating reverse osmosis) should be piped through a circulated system, connected to dedicated faucets at sinks in labs (for use for general rinsing, incubator fill, water baths etc.), and also connected to polishers for dispense directly from the polisher system of the required grade of ultrapure water. Polisher outlets should not be piped to sink taps or other remote outlets unless the arrangement includes a point of use ultrafilter

and a circulation arrangement (preferably back through 254nm UV) to maintain the intended water quality.

The inconsistency amongst standards, process variations, and diversity of applications can leave questions as to appropriate feed water qualities for central systems. Polisher manufacturers sometimes have feedwater demands that are not practically realistic for central building systems, or even established as scientifically necessary for many applications. For example, it is not uncommon to find manufacturers requesting TOC levels as low as 50 ppb for feedwater sources; significantly escalating criteria that was commonly specified as high as 1 to 2 ppm or even ignored by many manufacturers just a few years ago! Some of these qualities are rarely achieved, and even if recognized, there may be more cost-effective approaches. A realistic approach to producing and maintaining quality feedwater is necessary to ensure operating economies and flexible laboratories. Quality of input water does correlate to output water quality and component service frequency. Table 5 summarizes feed water quality requirements for typical high-end polishers for production of ultra-pure water.

**TABLE 5, COMPARISON OF MANUFACTURERS RECOMMENDED FEEDWATER SPECIFICATIONS FOR ULTRAPURE WATER POLISHERS**

| Product/Parameter | Elga Labpure UltraGenetic | Sartorius "Arium-Pro" | Thermo-Fisher Barnstead Genpure | Thermo-Fisher Barnstead Nanopure |
|---|---|---|---|---|
| **Specific Conductivity Resistivity /(25°C)** | (1MΩ-cm for DI feed), >0.33MΩ-cm resistivity for RO Feed | <100 µS conductivity (>.01MΩ-cm resistivity) | <2 µS conductivity (>0.5MΩ-cm resistivity) | NS |
| **TOC** | <50ppb | <50 ppb | <50ppb | <1ppm |
| **Turbidity** | NS | < 1 NTU | <1 NTU | <1 NTU |
| **pH** | NS | 4 to 10 | NS | NS |
| **Pre-treatment Method** | RO, DI, Distilled | RO, DI, Distillation | RO, DI, Distillation | RO, DI, Distillation |
| **Silt Density Index (Colloids)** | 1.0, Additional 2 µm filter recommended if water not treated by RO | NS | 1.0, Additional 1µm filter recommended if water not treated by RO. | 5 |
| **Silicate** | <2ppm | NS | <2 ppm | <1 ppm |
| **CO2** | <30ppm | <30 ppm per manuf. | <30 ppm | NS |
| **Bacteria** | | NS | <100 CFU/ml | NS |
| **Free Chlorine** | <.05ppm | NS | <.05ppm | NS |
| **TDS (CaCO3)** | | NS | NS | <70 ppm |

An example of a quality feed water system, suitable for a wide range of applications and polisher feeds might exhibit the attributes as indicated in Table 6, or as indicated in ASTM D1193 for Type 3 or Type 2 water, with a microbial qualification (B or C):

**TABLE 6, RECOMMENDED PRACTICAL PURIFIED FEED WATER QUALITY PARAMETERS AND ASSOCIATED MONITORING LEVELS FOR GENERAL FEED-WATER SERVICE TO POLISHERS AND LAB SINKS**

| Parameter | Target | Limit | Alert Level | Action Level |
|---|---|---|---|---|
| Resistivity (min/max), (25°C) | 1 to 5 MΩ cm | 1 to 5 MΩ cm | <1 MΩ cm, >5MΩ cm | <0.5 MΩ cm >5MΩ cm |
| TOC (max) | <200 ppb | 500 ppb | >200 ppb | >500 ppb |
| Microbial | <10 CFU/ml | 100 CFU/ml | >50 CFU/ml | >100 CFU/ml |
| Endotoxin | <5 EU/ml | <25 EU/ml | 10 EU/ml | >25 EU/ml |

Localized production of required grades of water direct from tap water sources may sometimes be desirable, however the lack of flexibility, maintenance requirements, needs for multiple grades of water, as well as on-going operational costs and spatial implications often favor the use of central systems with localized polishers. Point-of-use treatment systems should be considered to meet pure water requirements if the application needs are remote or uniquely specialized compared to bulk water needs.

## Monitoring Water Purity

Since inorganic salts and dissolved organics are the major contaminants that affect most laboratory applications it is important that they are monitored on-line in laboratory water systems. The key rapid, on-line techniques are resistivity and TOC (though TOC is also sometimes measured off-line). An on-line conductivity sensor with a display unit that can compensate for temperature is used to measure resistivity and conductivity. pH is not an effective measurement to determine water purity because of the rapid reactivity of water with its surroundings. Also, water has a low conductance, which causes instability in most pH meters.

Other monitoring methods are required to measure the presence or concentration of non-ionized chemicals and sub-ppb concentrations of ions. These may include inductively coupled plasma mass spectrometry, ion chromatography and graphite furnace atomic absorption spectrometry (GFAAS) (also known as Electrothermal Atomic Absorption Spectrometry (ETAAS))

Total Organic Carbon (TOC) is used to detect organics although they cannot all be measured routinely. TOC methodologies oxidize the organic substances in water samples and then measure the resultant oxidation products. They then measure either the CO2 or acid by product or the change in conductivity due to all the oxidized species. The main role of TOC is for monitoring and trending water quality. Trend monitoring provides a way to anticipate maintenance. It is important to collect regular quality control trend data and follow the manufacturer's maintenance recommendations for sanitation.

Bacterial contamination can be monitored in several ways. A sample of water can be centrifuged and the sediment stained and observed microscopically. Alternatively a fluorescence microscope which is an optical microscope that uses fluorescence and phosphorescence instead of, or in addition to, reflection and absorption to study properties of organic or inorganic substances can be applied to a stained filtered sample to better quantify the bacterial count. The traditional way to monitor bacterial contamination is to plate a sterile 0.22 µm membrane through which the water has been filtered on the surface of a low nutrient media and incubated for 3 to 5 days and count the colony forming units. It is important to recognize the limitations of plate count methods in their detection only of viable bacteria, and to recognize the importance of sufficient sampling quantity and locations. Many bacteria in purified water systems will not be free-floating, but instead within biofilms. Plate count methods often substantially underestimate microbial quality of water systems, and can be well supplemented by endotoxin monitoring.

To monitor for bacterial endotoxins levels a standard test based on Limulus Amebocyte Lysate activity can be utilized. RNase, DNase and proteases detection kits are also available for off line testing of these contaminants. For less critical applications, gel clot methods may be adequate. An advantage of endotoxin monitoring is the rapidness with which results can be obtained, as compared for example to HPC.

## Water applications

In the course of conducting experiments and filling equipment, as with all reagents and methodologies, fitness for use validations must be appropriately conducted by the researcher to the extent necessary to ensure the integrity of results. The extent and methodology by which this is accomplished can vary with the application; however guidance is available following typical standards for validation (such as cGMP/cGLP, as well as published guidance available in polisher equipment validation manuals, and standards such as produced by ASTM and CLSI, and ACS).

1. ASTM Type 1 and CLSI SRW waters are of the highest quality, but must be appropriately specified for end use requirements. Such ultrapure waters are generally used for the most critical applications – including HPLC and trace analysis.

2. ASTM Type II RGW and CLSI CLRW waters are often suitable for preparing culture media, and for many microbiology and bacteriology procedures. It is particularly important that sources of microbial contamination for these uses be carefully monitored to prevent contamination.

3. ASTM Type III RGW and CLSI Instrument Feed Water is generally suitable for glass washing, incubator/humidity cabinets, polisher feed for preparation of ultrapure water, and similar applications.

4. ASTM Type IV RGW is sometimes used for glassware washing, cooling applications, etc. ThermoScientific recommends the following RGW for general lab, analytical and life science applications (Table 7) (ThermoScientific, 2009).

**Table 7**

| | Type of Water | | | |
|---|---|---|---|---|
| **Application and Interest Areas** | **Ultrapure Type 1** | **Pure Type 2** | **RO** | **Cartridge and Filter Systems** |
| **General Lab Purpose** | | | | |
| Autoclave | | X | X | X |
| Humidification | | X | X | X |
| Glassware Washing/Rinsing | | X | X | X |
| General Lab Equipment (water baths, incubators, etc.) | | X | X | X |
| Feed Water to Type 1 Systems | | X | X | |
| Media Prep | | X | | |
| Buffer Prep | | X | | |
| Chemical and Biochemical Reagent Prep | | X | | |
| **Analytical** | | | | |
| High Performance Liquid Chromatography (HPLC) | X | | | |
| Gas Chromatography (GC) | X | | | |
| Ion Chromatography (IC) | X | | | |
| Inductively Coupled Plasma Spectroscopy (ICP) | X | | | |
| Mass Spectroscopy (MS) | X | | | |
| Atomic Absorption (AA) | X | | | |
| Total Organic Carbon (TOC) | X | | | |
| **Life Sciences** | | | | |
| Genomics (ex. PCR, Mutagenesis) | X | | | |
| Proteomics (ex. Crystallography, Electrophoresis) | X | | | |
| Immunology (ex. Monoclonal Antibody Production, Blots) | X | | | |
| Pharmacology | X | | | |
| Cell and Tissue Culture | X | | | |
| Drug Discovery | X | | | |

"ELGA Pure LabWater Guide, An essential overview of lab water purification applications, monitoring and standards" provides recommendations for analytical and general applications (Table 8). (ELGA, n.d.)

**TABLE 8**

## Analytical and general applications

| Technique | Sensitivity | Resistivity* MΩ-cm | TOC ppb | Filter µm | Bacteria CFU/ml | Endotoxin EU/ml | Nuclease | Grade of Pure Water |
|---|---|---|---|---|---|---|---|---|
| Electrochemistry | General | >5 | <50 | <0.2 | NA | NA | NA | General lab |
| | High | >18 | <10 | <0.2 | <1 | NA | NA | Ultra pure |
| Feed to stills | Low | >0.05 | <500 | NA | NA | NA | NA | Primary |
| Feed to ultra pure water systems | General | >0.05 | <50 | NA | NA | NA | NA | Primary |
| | High | >1 | <10 | <0.2 | <1 | NA | NA | Ultra pure |
| Flame-AAS | General | >5 | <500 | <0.2 | NA | NA | NA | General lab |
| GC-MS | High | >18 | <3 | <0.2 | <1 | NA | NA | Ultra pure |
| General chemistry | General | >1 | <50 | <0.2 | <10 | NA | NA | General lab |
| GF-AAS | High | 18.2 | <10 | <0.2 | <10 | NA | NA | Ultra pure |
| Glassware washing | General | >1 | <50 | <0.2 | <10 | NA | NA | General lab |
| | High | >18 | <10 | <0.2 | <1 | NA | NA | Ultra pure |
| HPLC | General | >1 | <50 | <0.2 | <1 | NA | NA | General lab |
| | High | >18 | <3 | <0.2 | <1 | NA | NA | Ultra pure |
| ICP-AES | General | >5 | <50 | <0.2 | NA | NA | NA | General lab |
| | High | >18 | <10 | <0.2 | <1 | NA | NA | Ultra pure |
| ICP-MS | General | >10 | <50 | <0.2 | <10 | NA | NA | General lab |
| | High | 18.2 | <10 | <0.2 | <1 | NA | NA | Ultra pure |
| Ion chromatography | General | >5 | <50 | <0.2 | <10 | NA | NA | General lab |
| | High | 18.2 | <10 | <0.2 | <1 | NA | NA | Ultra pure |
| Sample dilution and reagent preparation | General | >1 | <50 | <0.2 | <1 | NA | NA | General lab |
| | High | >18 | <10 | <0.2 | <1 | NA | NA | Ultra pure |
| Solid phase extraction | General | >1 | <50 | <0.2 | <10 | NA | NA | General lab |
| | High | >18 | <3 | <0.2 | <1 | NA | NA | Ultra pure |
| Spectrophotometry | General | >1 | <50 | <0.2 | <1 | NA | NA | General lab |
| | High | >18 | <10 | <0.2 | <1 | NA | NA | Ultra pure |
| Steam generation | General | >1 | <50 | <0.2 | <1 | NA | NA | General lab |
| TOC analysis | General | >1 | <50 | <0.2 | <10 | NA | NA | General lab |
| | High | >18 | <3 | <0.2 | <1 | NA | NA | Ultra pure |
| Trace metal detection | General | >5 | <50 | <0.2 | <10 | NA | NA | General lab |
| | High | 18.2 | <10 | <0.2 | <1 | NA | NA | Ultra pure |
| Water analysis | General | >5 | <50 | <0.2 | <10 | NA | NA | General lab |
| | High | >18 | <10 | <0.2 | <1 | NA | NA | Ultra pure |

* At 25°C NA - *not applicable* ND - *not detected* Figures in red - *critical impurities*

"ELGA Pure LabWater Guide, An essential overview of lab water purification applications, monitoring and standards" provides recommendations for Life Science applications (Table 9) (ELGA, n.d.).

**TABLE 9**

## Life science applications

| Technique | Sensitivity | Resistivity* MΩ-cm | TOC ppb | Filter µm | Bacteria CFU/ml | Endotoxin EU/ml | Nuclease | Grade of Pure Water |
|---|---|---|---|---|---|---|---|---|
| **Bacterial cell culture** | General | >1 | <50 | <0.2 | <1 | NA | NA | General lab |
| **Clinical biochemistry** | USP/EP<br>CLSI | >2<br>>10 | <500<br><500 | <0.2<br><0.2 | <1<br><1 | NA<br>NA | NA<br>NA | General lab<br>General lab |
| **Electrophoresis** | High | >18 | <10 | UF | **<1** | **<0.005** | **ND** | Apyrogenic Ultra pure |
| **Electrophysiology** | General | >1 | <50 | <0.2 | <1 | NA | NA | General lab |
| **ELISA** | General | >1 | <50 | <0.2 | <1 | NA | NA | General lab |
| **Endotoxin analysis** | Standard<br><br>High | >1<br><br>>18 | <50<br><br><10 | <0.2<br><br>UF | <1<br><br>**<1** | <0.05<br><br>**<0.002** | NA<br><br>**ND** | Apyrogenic Lab<br>Apyrogenic Ultra pure |
| **Histology** | General | >1 | <50 | <0.2 | <1 | NA | NA | General lab |
| **Hydroponics** | General | >1 | <50 | <0.2 | <1 | NA | NA | General lab |
| **Immunocytochemistry** | High | >18 | <10 | UF | **<1** | **<0.002** | **ND** | Apyrogenic Ultra pure |
| **Mammalian cell culture** | High | >18 | <10 | UF | **<1** | **<0.002** | **ND** | Apyrogenic Ultra pure |
| **Media preparation** | General | >1 | <50 | <0.2 | <1 | NA | NA | General lab |
| **Microbiological analysis** | General | >1 | <50 | <0.2 | <1 | NA | NA | General lab |
| **Molecular biology** | High | >18 | <10 | UF | **<1** | **<0.002** | **ND** | Apyrogenic Ultra pure |
| **Monoclonal antibody research** | General<br>High | >1<br>>18 | <50<br><10 | <0.2<br>UF | <1<br>**<1** | NA<br>**<0.002** | NA<br>**ND** | General lab<br>Apyrogenic Ultra pure |
| **Plant tissue culture** | High | >18 | <10 | UF | **<1** | **<0.002** | **ND** | Apyrogenic Ultra pure |
| **Radioimmunoassay** | General | >1 | <50 | <0.2 | <1 | NA | NA | General lab |

* At 25°C NA - *not applicable* ND - *not detected* Figures in red - *critical impurities*

## Summary

For scientific and economic reasons, research, medical, and design professionals should become familiar with and apply the water grade most applicable to their needs, and must perform appropriate fitness for use validations. Reagent grade water specifications have been described by standard setting organizations such as ASTM, ISO 3696 and CLSI®-CLRW- SRW but the water qualities published by each organization varies. The four reagent grades of water identified by ASTM are Types I, II, III, & IV with Type I being the most pure, often referred to as ultrapure. It is usually necessary to employ several techniques in an integrated system to achieve the required water purity level, and specific water quality parameters are ultimately user-defined. Well-designed central systems of moderate water quality are highly flexible and should be used with point of use polishers to achieve flexible, economical operation. Systems must be well maintained including controls over materials, equipment connections, service frequency and piping arrangements to protect water quality. All water purification systems start with potable water that meets Federal Standards, and is treated or polished by a combination of techniques to achieve the desired purity for the specific application.

The Division of Technical Resources (DTR), Office of Research Facilities (ORF) has published special water requirements for animal drinking water that are available at (Animal Drinking Water - Part 1 November 2012) and (Animal Drinking Water - Part 2 December 2012). Water requirements for aquatic species can be found at (Aquatic Facilities Think Beyond The Guide Part ll) and purified laboratory water requirements for NIH facilities in general can be found in the most current NIH Design Requirements Manual (DRM) at:

Policies and Guidelines Biomedical and Animal Research Facilities Design Policies and Guidelines Pages

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