ELGA VEOLIA

Water

The Essence of the Lab

How to make effective use of your most fundamental reagent

Water

The Essence of the Lab

Contents

1. *Water: How much do you really know about your most important reagent?*

As anyone that has spent any length of time working in a lab will know, scientists make use of water in a plethora of processes throughout the day, from HPLC and cell culture through to buffer preparation and wiping down the bench. As such, they get through a lot of it. A typical laboratory is estimated to use around five-times as much water as a comparably sized office block – that's about 35 million liters per year.[1]

What's especially interesting is how little many scientists actually know about this essential lab reagent. Have you ever considered the level of purity of your standard water supply? Or have you just grabbed the distilled or deionized water next to your bench without really considering the implications of using the wrong level of purity? If so, what you actually end up using may not be what you really need!

When you consider that approximately 70% of HPLC performance problems are thought to be directly attributable to water quality and water impurities can affect most enzymatic reactions that you rely upon in molecular biology, you quickly begin to realize that water purity is something that we as scientists should be paying more attention to.

By making use of the correct level of water purity for your experiments you are more able to produce consistent and accurate results, free of avoidable errors. That means better data quality, more reliable conclusions and downstream analyses you can trust.

In the pages that follow, we discuss the differences in water purity levels, along with the technologies available for defining and assessing water purity. We also include an application-orientated table for ease of matching water purity levels with each individual application. Lastly, we cover some of the more practical considerations such as space requirements, the effects of storing pure water for prolonged periods of time and the future of water purification in the lab.

Ultimately, the success of your work in the lab hinges on the quality of your reagents and the reliability of your experiments. We'll show you how to make sure your water supply delivers the performance you need to produce results.

2. *Water purity is important for every lab application*

Being able to produce reliable data from your experiments is dependent upon the quality of the materials you use: poor reagents in, means poor results out. It becomes almost second nature to think about using aseptic techniques, for example, when conducting microbiology or molecular biology to avoid contamination at the bench, but we seldom apply this rigorous approach to ensuring the water we use is of a similarly high standard.

Water purity can have marked effects on your data due to any number of possible contaminants or ionic imbalances, the likes of which you may not have considered before. Here are a few examples.

2.1 Blotting techniques

Whether you're analyzing DNA, RNA or proteins you're likely to be making use of Southern, Northern or Western blotting techniques. The successful outcome of these procedures depends upon the water quality used for sample and solution preparation.

For example, Western blotting can be compromised by the addition of various proteins via bacterial contamination. Bacteria can also release nucleases causing degradation of nucleic acid samples during Northern and Southern blotting, processes which are also at further risk from other organic molecules often found in the water. These organic molecules can interfere with the hybridization process when stray negatively charged molecules bind non-specifically in place of DNA or RNA, upsetting the hybridization procedure.

Finally, excessively high ionic contamination can disrupt migration during electrophoresis as a result of an altered global charge in the solution. For all these reasons, maintaining an adequate level of water purity is paramount for producing consistent technical performance and reliable results.

2.2 Chromatography

High performance liquid chromatography (HPLC) provides users with a high degree of sensitivity when determining minor and major components of complex mixtures. When you're looking to detect in the parts per billion (ppb) ranges, the presence of impurities can be a major confounding factor *(Figure 1)*. Unsurprisingly, good chromatographic performance is highly dependent upon the purity of the water used.[3]

Organic compounds will likely be the most influential water contaminant, as they compete with the analyte in the mobile phase to reduce the effective levels of analyte retained in the column, with a resulting reduction in sensitivity. Other concerns include bacteria, which can form blockages and influence the process via organic by-products, and ionic contaminants which can affect some chromatographic separations.

Ion chromatography (IC) is still used in many labs, due to its relative simplicity, excellent reliability and broad applicability in areas such as bioanalysis, geochemistry and a wide variety of other research areas and industries. When using IC, trace levels of ions can have a much more pronounced effect than with HPLC (*Figure 2*) and can result in inaccurate results on account of distorted calibration and/or blank signals.

Dissolved gases in the water can alter the pH of the mobile phase (for example, carbon dioxide will produce carbonic acid when dissolved), which in turn can alter elution times or reduce the effective capacity of the stationary phase. As with HPLC, bacteria and organic compounds can have detrimental effects by producing higher background and/or blank values, while the bacteria themselves can release ions which may interfere with the analysis. Finally, particles and colloids can result in an elevated back column pressure, which may affect pumps and thus impact on the overall integrity of the system.

Figure 1. A comparison of purified water qualities used in HPLC. 50 ml water concentrated on a C18 column and eluted with a water:acetonitrile gradient, 0–100% at 5%/min, flow rate 2 ml/min, with UV detection at 254 nm.[4]

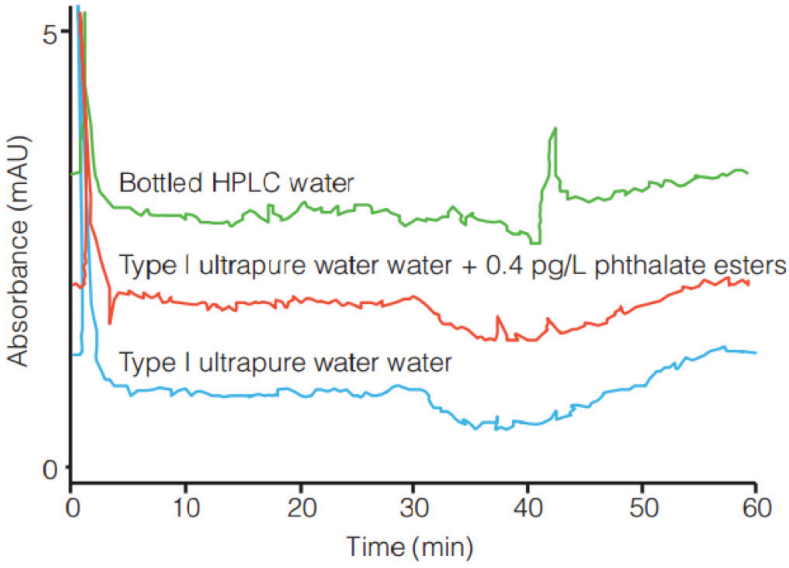
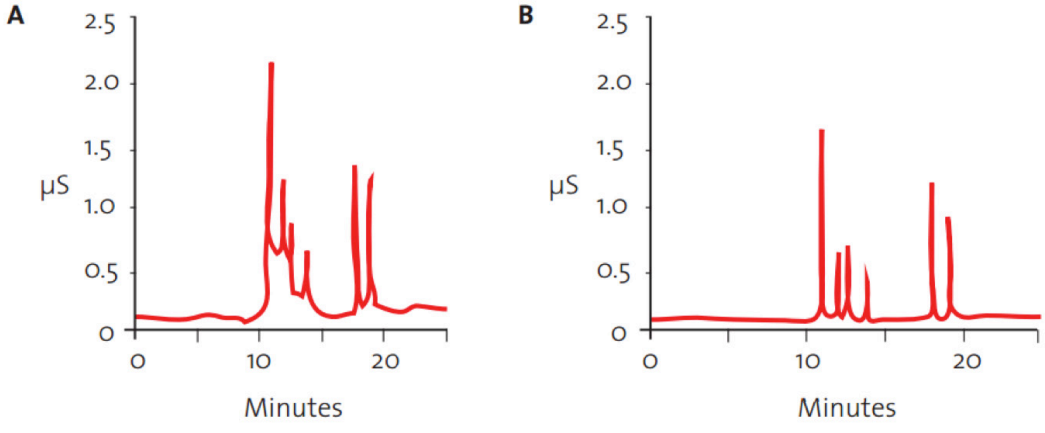


Figure 2. The effect of ionic contaminants from water on baseline used in ion chromatography, integration and resolution: (A) poor water quality and (B) Type I (18.2 MΩ-cm) water. The 'noise' introduced around the 10–15 minute mark is clearly apparent.[5]



2.3 Spectroscopy and spectrometry

If your experimental approaches call for the use of either spectroscopy or spectrometry, it's likely that a high level of water purity is essential. These techniques are highly sensitive and can be costly to carry out, so ensuring your results are as accurate as possible in every run should be a key goal of any good analyst.

Performing inductively coupled plasma mass spectrometry (ICP-MS), for example, demands that your solutions have virtually zero levels of additional elements or ions. With detection resolutions down into the parts per trillion (ppt), impurities can lead to artificially high sample concentrations or errors in blanks and calibration samples, meaning the accuracy of your results will be severely affected.

2.4 Polymerase chain reaction

Water quality isn't just important for high-grade analytical instruments, it also affects one of the most frequently used techniques in molecular biology and genetics labs: the polymerase chain reaction (PCR). PCR is wholly dependent upon the action of DNA polymerases, enzymes that amplify single stranded DNA 'templates' to produce a vast number of additional copies.

Although fairly robust, the PCR process can still be inhibited relatively easily by water contaminants. For example, nucleases are enzymes that will cleave the phosphodiester linkages between nucleotides, severely disrupting the PCR reaction to essentially leave you without a stable product. Therefore, nuclease-free water is something that most molecular biologists consciously utilize.

However, nucleases are not the only danger: bacteria in the water will also lead to erroneous results – nobody wants to risk completing a series of experiments only to realize that they've been inadvertently amplifying lengths of contaminating bacterial DNA!

Non-biological contaminants can also affect PCR: DNA polymerases are highly sensitive to various common cations (e.g. Cu^{2+}, Fe^{2+}, Ni^{2+}, etc.), which can disrupt substrate binding and inhibit enzyme activity. Negatively charged organic compounds can also compete with DNA at the polymerase active site, resulting in inefficient catalysis of the reaction, all of which can disrupt experiments.

2.5 Histology and immunohistochemistry

The importance of water quality is perhaps less obvious in the cases of histology and immunohistochemistry (IHC). However, impurities can still lead to poor quality data and unreliable conclusions. For example, bacterial contamination can lead to the introduction of artifacts in mounted samples as a result of the bacteria adhering to tissue sections. Bacteria can also release alkaline phosphatase (AP), which can interfere with IHC protocols that make use of AP for chromogenic detection.

At the molecular level, several metal ions can cause unwanted precipitation reactions when at high enough concentrations in staining solutions, or even interfere with antibody-antigen binding reactions when performing IHC. As such, water quality is often a key area to troubleshoot when IHC experiments are not performing as expected.

3. *Everything you need to know about water impurities*

Whatever type of experiment or reaction you are undertaking, lack of appropriate water purity can adversely affect your results. However, what are these impurities and how do we assess and subsequently classify water based on them?

Almost all of the water you use in the lab starts off as regular drinking/tap water, regardless of final purity. This water initially comes from natural sources, such as rivers or underground aquifers, before undergoing a series of steps to render it suitable for drinking.

Raw water can itself vary in composition depending on the season or geography. Underground water, for example, is high in salts and low in organic content, whereas water from surface-derived sources is of a more intermediate quality, but may contain chemicals from agricultural runoff or similar sources.

The process of taking raw water and processing it into drinking water involves first screening for debris. The water is then clarified to remove suspended solids, gravity filtered through sand and then possibly subjected to ozone treatment. Granular activated carbon (GAC) may then be used to trap any dissolved organic matter, before the water is finally treated with chlorine to kill harmful microorganisms. An additional ultrafiltration step is used to remove *Cryptosporidium*.

This process was established to remove the various harmful impurities commonly found in raw water. Drinking water itself still contains numerous contaminants, many of which will distort scientific data as discussed earlier. These impurities mainly consist of dissolved inorganic compounds, factors such as calcium, magnesium and sodium salts, carbon dioxide, silicates, iron compounds, chlorides, aluminum, phosphates and nitrates. In addition, drinking water also contains dissolved organic compounds (mainly of biological origin), dissolved gases and microorganisms that have survived the ozonation and chlorine treatment.

3.1 Water impurities and their impact in the lab

There are several major classes of impurities to consider when evaluating the purity of your laboratory water.

Suspended particles

The most conspicuous contamination in any form of water is suspended matter. This can include anything from silt and vegetation through to colloids and pollutants, or even pathogens adsorbed onto other particles. Such factors can have obvious detrimental effects, for example, by blocking filters, chromatography columns or osmosis membranes.

Dissolved inorganic compounds

These make up the bulk of impurities found in water and are generally mineral in nature, typically existing as ions (e.g. sodium, iron, calcium, magnesium, manganese, nitrate, chloride, sulfate, and zinc). While many of these are naturally occurring, some, such as nitrates or salts, may be introduced anthropogenically from activities such as farming and road gritting. Ionic instability in labwater will affect both protein solubility and the successful formation of protein-protein and protein-lipid interactions, for example. This in turn can lead to alterations in enzymatic activity and therefore negatively impact on the overall rates of chemical reactions.

Dissolved organic compounds

These are principally of biological origin and come from three main sources: allochthonous, or terrestrial material from soil; autochthonous, or surface water-derived of algal or phytoplankton origin; and synthetic organic substances, of man-made or industrial origin.[6] Dissolved organic compounds can successfully support the growth of an array of microorganisms and therefore have the potential to severely confound biological experiments such as those involving eukaryotic and bacterial cell cultures. Sensitive processes such as chromatography will also be affected if dissolved organic compounds contaminate the eluents, reducing baseline stability and overall sensitivity. These compounds can also degrade other experimental targets, such as proteins and nucleic acids, reducing the reliability of your data.

Dissolved gases

Like dissolved inorganic compounds, dissolved gases can also upset ionic balance. For example, carbon dioxide will be absorbed in water to form carbonic acid, which can alter pH and reduce the capacity of anion exchange resins. In addition to this, the solubility of air in water will directly affect the concentrations of both oxygen and nitrogen, variables which can alter rates of certain biochemical reactions and impact on the reproducibility of your results. High concentrations of these dissolved gases can even result in bubble formation, the likes of which will disrupt spectrophotometric measures and can impede flow through micro-channels and columns.

Microorganisms

Microbial contamination represents a multifaceted problem in the lab: the bacteria themselves cause issues, but so do the compounds they release. Even worse, the aggregations they form can be the driving factor behind years of future contamination.

The presence of bacteria in labwater can spell disaster for those carrying out research or analysis where sterile conditions are essential. Bacteria can also affect biochemical reactions by competing with substrates at enzyme active sites, as well as having an effect at a much larger scale by blocking filters.

They also produce various compounds, most importantly nucleases and endotoxins. Nucleases will break down any target nucleic acids in your samples (such as DNA or RNA), while endotoxins represent a real threat to humans if they inadvertently find their way into the bloodstream, in addition to affecting cell culture and/or *in vitro* fertilization techniques.

Free-floating (planktonic) bacteria can also initiate the formation of biofilms on surfaces which, once attached, can continue to develop for several years. The bacterial colonies become encased in an extracellular polysaccharide matrix, which offers the bacteria protection and enhanced nutrient flow while also making their removal especially difficult. The presence of biofilms on lab surfaces represents an on-going source of contamination since the bacteria continue to be released in unpredictable bursts, along with the associated endotoxins and nucleases. Biofilm structures are a complication that you should tackle at the earliest possible stages of development in order to avoid unexpected levels of contamination and to preserve the integrity of your data.

3.2 How is water purity assessed and defined?

In order to implement a coherent classification system, we make use of several key factors describing the various properties of water.

Conductivity

This is reported as microSiemens per centimeter (µS/cm) at 25°C and is the reciprocal of resistivity *(see Table 1)*. It provides a measure of a fluid's ability to conduct electrical current. This is typically used when assessing water ranging from raw through to drinking water, and provides an important, non-specific indication of the level of ions in the water.

Resistivity

Reported as Mega-Ohms per centimeter (MΩ-cm) at 25°C, resistivity is related to conductivity: a high resistivity = a low conductivity. As such, it also provides a measure of the water's ionic content. Unlike conductivity, resistivity is primarily used in the assessment of ultra pure water.

Organic compound levels

Organic compounds can exist in water in numerous forms and so measuring each single one individually is impractical. Instead, the most useful indicator is considered to be the total organic carbon (TOC) content of the solution. This is measured via a process that oxidizes the organic compounds present and then quantifies the oxidation products generated. TOC is as close as we can currently get to a 'universal indicator' for the presence of organic impurities. Alternatively, chromatographic techniques may be employed to determine the specifics of organic content, but this is frequently considered both too expensive and time consuming to be used in general monitoring workflows.

Table 1. The relationship between conductivity and resistivity.

Conductivity (µS/cm)	Resistivity (MΩ-cm)
0.01	100*
0.055	18
0.1	10

*Theoretically predicted value.

Biological contamination

The presence of biological contaminants such as bacteria and other microorganisms is a common issue in untreated water. Bacterial levels, reported as colony forming units per milliliter (CFU/ml), are kept low via filtration, UV treatment and sterilant solutions. Following an incubation period in suitable growth media, individual bacterial species and total viable cell counts can be determined.

Bacteria counts may also be monitored through the use of epifluorescence testing in order to rapidly detect and distinguish between dead and living microorganisms. In addition to the bacteria themselves, endotoxins produced from the cell wall of gram-negative microorganisms (reported as endotoxin units per milliliter, EU/ml; 1 EU/ml approximately equal to 0.1 ng/ml) can be assessed using standard tests based on Limulus Amebocyte Lysate activity.

Presence of colloids

Suspended particles can cause water turbidity (measured in Nephelometric Turbidity Units, NTU) and are therefore filtered out of labwater as much as possible. This colloidal material is defined as being less than 0.5 µm in size and may contain iron, silica, aluminum or organic materials. The Fouling Index (FI) is frequently used to estimate the potential of water to block filters under 0.45 µm filter conditions.

3.3 Types of water

Depending on subsequent downstream processes, water purity can be classified into broad types, which define measurable physical and chemical properties *(Tables 2 and 3)*.

In addition to defining water according to the major types *(Table 2)*, the team at ELGA LabWater also makes use of four general grades of water specification:

1. **Primary grade water:** the lowest level of purity available, used primarily for feeding washing machines and rinsing labware not involved in critical processes.

2. **Deionized water:** multi-purpose, used in rinsing and making general lab solutions and has had the majority of its ions removed, with a resistivity of 1.0 –10.0 MΩ-cm.

3. **General laboratory water (Type III to II):** used in a broad range of applications from solution preparation to microbiological studies, with a resistivity of >1.0 MΩ-cm, TOC <50 ppb and bacterial count <200 CFU/ml.

4. **Ultra pure (Type I):** reaches the theoretical ideal levels of purity, with a resistivity of 18.2 MΩ-cm, TOC <10 ppb, bacterial count <10 CFU/ml. Endotoxins are also removed, and as such, ultra pure water typically contains <0.03 EU/ml, with nucleases and proteases at non-detectable levels.

Many labs will also make use of distilled and double distilled water, which has been produced by a slow, energetically intensive distillation process. Although this has the benefit of producing water with a long shelf life, it is still prone to re-contamination if stored for overly long periods of time.

Table 2. Types of water purity in descending levels of purity.[2]	
Type I+	Goes beyond the purity requirements of Type I water.
Type I	Often referred to as ultra pure, this grade is required for some of the most water-critical applications such as HPLC (High Performance Liquid Chromatography) mobile phase preparation, as well as blanks and sample dilution for other key analytical techniques including GC (Gas Chromatography), AAS (Atomic Absorption Spectrophotometry) and ICP-MS (Inductively Coupled Plasma Mass Spectrometry). Type I is also required for molecular biology applications, as well as other sensitive techniques such as mammalian cell culture and IVF (*In Vitro* Fertilization).
Type II+	Is the grade for general laboratory applications requiring higher than standard Type II water levels of inorganic purity.
Type II	Is employed for general laboratory use. This may include media preparation, the creation of pH solutions and buffers, and to feed certain clinical analyzers. It is also common for Type II systems to be used as a feed to a Type I system.
Type III	Is the grade recommended for non-critical work, which may include glassware rinsing, water baths, autoclave and disinfector feeds, as well as environmental chambers and plant growth rooms. These systems can also be used to feed Type I systems.

Table 3. Chemical and physical properties attributed to types of water.[8]

	Resistivity (MΩ-cm)	TOC (ppb)	Bacteria (CFU/ml)	Endotoxins (EU/ml)
Type I+	18.2	<5.0	<5.0	<0.03
Type I	>18.0	<10.0	<10.0	<0.03
Type II+	>10.0	<50.0	<50.0	N/A
Type II	>1.0	<50.0	<50.0	N/A
Type III	>0.05	<200.0	<200.0	N/A

3.4 Conforming to international standards

Around the world, several international boards have been established in order to generate consistent, high-quality standards across all industries. Some laboratories will also adopt standards outlined in the European, US or Japanese Pharmacopoeia. However, very few of these standards are specific to a particular application.

Clinical and Laboratory Standards Institute (CLSI) – formerly NCCLS

As of 2006 the CLSI has moved away from the typical Type I, II and III designations, instead preferring to request that water is 'fit for purpose', and only describing one grade in detail: Clinical Reagent Laboratory Water. Other standards from the CLSI include Special Reagent Water (SRW) and instrument feed water.

International Organization for Standardization (ISO)

The ISO based its specification on ISO 3696:1987 and has three grades of water: Grade 1, Grade 2 and Grade 3, where Grade 1 is the most pure *(see Table 4)*.

American Society for Testing and Materials (ASTM)

The ASTM uses D1193-06 and has four grades of water which cover the suitability of water for chemical analysis and physical testing *(see Table 5)*.

Table 4. Water quality parameters for ISO grades.

Parameter	Grade 1	Grade 2	Grade 3
pH value at 25°C	-	-	5.0–7.0
Conductivity (µS/cm) at 25°C, max	0.1	1.0	5.0
Oxidizable matter Oxygen content (mg/l), max	-	0.08	0.4
Absorbance at 254 nm and 1 cm optical path length, absorbance units, max.	0.001	0.01	-
Residue after evaporation on heating at 110°C (mg/kg), max	-	1	2
Silica (SiO_2) content (mg/l), max	0.01	0.02	-

Table 5. Water quality parameters for ASTM types.

Parameter	Type I*	Type II**	Type III***	Type IV
Conductivity (µS/cm) at 25°C, max	0.056	1.0	0.25	5.0
Resistivity (MΩ-cm) at 25°C, max	18.0	1.0	4.0	0.2
pH at 25°C	-	-	-	5.0–8.0
TOC (µg/l), max	50	50	200	No limit
Sodium (µg/l), max	1	5	10	50
Silica (µg/l), max	3	3	500	No limit
Chloride (µg/l), max	1	5	10	50

*Requires use of 0.2 µm membrane filter; **Prepared by distillation; ***Requires the use of 0.45 µm membrane filter.

As a scientist, maintaining a high level of accuracy is one of your primary goals, so you can be sure of generating data that can be trusted. Fortunately, the potential impact of water impurities represents an avoidable threat, if appropriately considered. A range of technologies exist for purifying water, each with their own advantages and disadvantages. In this section we explore the processes behind water purification and provide advice for carrying it out cost- and time-effectively.

4.1 Water pretreatment technologies

Before moving straight on to generating water of the highest possible purity, it is worth considering the benefits offered by water pretreatment; by making use of relatively simple and high volume pretreatment methods, you can effectively enhance subsequent steps and keep costs down.

One pretreatment system, the use of microporous depth filters, provides an effective physical barrier to many types of contaminant. Made from compressed fibers of materials through which the water passes, these microporous depth filters trap or adsorb particles of a nominal size.

This process can be enhanced by the use of specialized surfaces, which can attract and retain the naturally occurring charged colloids even if they are smaller than the specified pore size. The pores in depth filters typically range from 1–50 µm and remove greater than 98% of suspended solids.

Activated carbon (AC), produced by pyrolysing coconut shells or coal at 800–1000^{0}C in the presence of water and carbon in a kiln, is used to remove chlorine and chloramine, the likes of which can damage the filters and membranes used further along in the purification process. AC catalytically breaks down chlorine and absorbs organics. Both AC and microporous depth filters need to be replaced frequently to maintain optimal levels of performance.

4.2 Technologies for increasing water purity

After the pretreatment stage there are several additional, more complex, technologies that you can utilize to further increase water purity.

Reverse Osmosis (RO)

RO makes use of specialized, semi-permeable membranes that remove particles greater than 1 nm in diameter and typically over 95% of the ionic and organic contamination. RO works by feeding water under pressure to the membrane. Some of the water crosses the membrane (the permeate) and some is left behind containing the majority of impurities (the concentrate), which can then be discarded. Pretreatment ensures that the membrane used at this later stage is not damaged by chlorine and chloramines or blocked by particulates and colloids. Overall, water recovery from a laboratory RO system is typically between 15–30% permeate, but it can be pushed as high as 75% depending on the feed water composition and pretreatment processes. While cost-effective, RO does not remove dissolved gases and element types have to be operated under limited flow rates. It also requires good pretreatment in order to avoid unwanted problems like fouling (organic/colloid deposits) and piercing (physical damage caused by larger particles). However, it is very effective at removing almost all forms of contamination, is a relatively easy process to monitor and requires minimal maintenance.

Ion exchange (IX)

Beds of IX resins are composed of sub-1 mm porous beads and can be purchased as cartridges or cylinders. These beds function by removing ions from the water by exchanging them for H^+ and OH^- ions. The beads are made from cross-linked insoluble polymers with many ionic exchange sites, and are themselves either anionic or cationic. Ions within the water compete for a place at the exchange sites based on their charge density. However, this process has no effect on reducing bacteria, organics or particulates, has a capacity limited by the number of free IX sites and requires high quality pretreated water so as not to prematurely exhaust the resins. The resins themselves must be replaced or regenerated regularly. On the other hand, the process is both inexpensive and removes all dissolved ionic compounds (<1 ppb total ionic contamination). It also allows for on-demand filtration options. As such, it is most effective when combined with a purification method removing larger contaminants, such as EDI (see electrodeionization below).

Electrodeionization (EDI)

EDI combines features of both IX and RO in order to produce a higher level of purity than the individual processes alone are capable of. Water typically passes through RO membranes and IX resins under the force of an applied voltage. This process still requires good quality feed water and removes only a limited number of charged organics, but it also removes dissolved ions giving a resistivity of 5–17 MΩ-cm and a TOC below 20 ppb. It also has the benefit of having continuously regenerating resins that are not exhausted like regular IX resin beds, thus avoiding the sudden release of boron, silicon and organic contaminants as a cartridge exhausts.

Filtration

This method is usually performed using microfiltration (MF) and ultrafiltration (UF) systems, both of which are carried out after the initial RO process. Both systems employ a physical barrier to contamination and are defined according to the size of the contaminant(s) they each remove. MF systems typically remove factors between 0.05–0.22 µm in size and are frequently operated as close as possible to the point-of-use.

MF is very useful in applications such as HPLC where it can remove colloids, bacteria and particulates. UF is much the same in operation as MF, but uses a pore size typically within the 0.001–0.1 µm range. UF is used in applications such as PCR since it removes nucleases and endotoxins as well as organics, bacteria and particulates.

Ultraviolet (UV) light

People have been capitalizing on the bactericidal effectiveness of shortwave UV light (UV-C) since the 1900s.
The UV light is utilized in tandem with flowing water. In addition to controlling bacteria, UV light can also oxidize organic compounds, reducing TOC to <5 ppb depending on the wavelength.

Distillation

Distilled water has a long and established history. It is produced by heating water until it converts into a gaseous state, before cooling it again so that it condenses back into a liquid. This transition separates the water from its contaminants, barring those with vapor pressures close to that of water. Distilled water possesses a long shelf life but prolonged storage can eventually lead to re-contamination.

Degassing

Degassing is a process that uses a hydrophobic membrane filter to remove gases such as carbon dioxide and oxygen. This is achieved by passing the water down one side of the membrane, with a vacuum or flush gas on the other side. The reduced pressure on the non-liquid side encourages gases within the water to leave and move across the membrane. This can produce water with dissolved carbon dioxide levels as low as 1 mg/l.

Vent filters

Vent filters can be fitted to a reservoir to prevent contaminants from entering stored water. They are comprised of microporous filters which operate by virtue of the filter's hydrophobic nature. By combining these with absorptive materials, a composite vent filter (CVF) will minimize carbon dioxide and organic contamination, as well as airborne particulates.

5. *Selecting the right water purity for your application*

With such a variety of water purification techniques available and a plethora of endpoint parameters to measure, it can sometimes be challenging to match your application with the recommended level of water purify. To help you do this quickly and effectively, we have compiled a list of common applications and the water purity they require in Table 6.

Table 6. Scientific application and recommended water purity parameters.

Technique	Application sensitivity required	Resistivity (MΩ-cm)*	TOC (ppb)	Filter (µm)	Bacteria (CFU/ml)	Endotoxin (EU/ml)	Nuclease	Water grade	Water type
Feed to still	Low	>0.05	<500	NA	NA	NA	NA	Primary	III
Feed to ultra pure water system	General	>0.05	<50	NA	NA	NA	NA	Primary	II+
	High	>1	<10	<0.2	<1	NA	NA	Ultra pure	I
General chemistry	General	>1	<50	<0.2	<10	NA	NA	General lab	II+
Glassware washing	General	>1	<50	<0.2	<10	NA	NA	General lab	II+
	High	>18	<10	<0.2	<1	NA	NA	Ultra pure	I
Media preparation	General	>1	<50	<0.2	<1	NA	NA	General lab	II+
Solution preparation and Dilutions	General	>1	<50	<0.2	<1	NA	NA	General lab	I
	High	>18	<10	<10	<1	NA	NA	Ultra pure	I
Steam generation	General	>1	<50	<0.2	<1	NA	NA	General lab	I
Bacterial cell culture	General	>1	<50	<0.2	<1	NA	NA	General lab	I
Clinical biochemistry	USP/EP	>2	<500	<0.2	<1	<1	NA	General lab	I
	CLSI	>10	<500	<0.2	<1	<1	NA	General lab	I
Electrophoresis	High	>18	<10	UF	<1	<0.005	ND	Apyrogenic Ultra pure	I
Electrophysiology	General	>1	<50	<0.2	<1	NA	NA	General lab	I
ELISA	General	>1	<50	<0.2	<1	NA	NA	General lab	I
Endotoxin analysis	Standard	>1	<50	<0.2	<1	<0.05	NA	Apyrogenic General lab	I
	High	>18	<10	UF	<1	<0.002	ND	Apyrogenic Ultra pure	I
Histology	General	>1	<50	<0.2	<1	NA	NA	General lab	I
Hydroponics	General	>1	<50	<0.2	<1	NA	NA	General lab	I
Immunocyto-chemistry	High	>18	<10	UF	<1	<0.002	ND	Apyrogenic Ultra pure	I

Continued overleaf

Table 6 (continued). Scientific application and recommended water purity parameters.

Technique	Application sensitivity required	Resistivity (MΩ-cm)*	TOC (ppb)	Filter (µm)	Bacteria (CFU/ml)	Endotoxin (EU/ml)	Nuclease	Water grade	Water type
Mammalian cell culture	High	>18	<10	UF	<1	<0.002	ND	Apyrogenic Ultra pure	I
Microbiological analysis	General	>1	<50	<0.2	<1	NA	NA	General lab	I
Molecular biology	High	>18	<10	UF	<1	<0.002	ND	Apyrogenic Ultra pure	I
Monoclonal antibody research	General High	>1 >18	<50 <10	<0.2 UF	<1 <1	NA <0.002	NA ND	General lab Apyrogenic Ultra pure	I I
Plant tissue culture	High	>18	<10	UF	<1	<0.002	ND	Apyrogenic Ultra pure	I
Radioimmuno-assay	General	>1	<50	<0.2	<1	NA	NA	General lab	I
Electrochemistry	General High	>5 >18	<50 <10	<0.2 <0.2	NA <1	NA NA	NA NA	General lab Ultra pure	II I
Flame-AAS	General	>5	<500	<0.2	NA	NA	NA	General lab	II
GC-MS	High	>18	<3	<0.2	<1	NA	NA	Ultra pure	I
GF-AAS	High	18.2	<10	<0.2	<10	NA	NA	Ultra pure	I+
HPLC	General High	>1 >18	<50 <3	<0.2 <0.2	<1 <1	NA NA	NA NA	General lab Ultra pure	II I
ICP-AES	General High	>10 18.2	<50 <10	<0.2 <0.2	<10 <1	NA NA	NA NA	General lab Ultra pure	II+ I+
ICP-MS	General High	>10 18.2	<50 <10	<0.2 <0.2	<10 <1	NA NA	NA NA	General lab Ultra pure	II+ I+
Ion chromatography	General High	>5 18.2	<50 <10	<0.2 <0.2	<10 <1	NA NA	NA NA	General lab Ultra pure	II+ I+
Solid phase extraction	General High	>1 >18	<50 <3	<0.2 <0.2	<10 <1	NA NA	NA NA	General lab Ultra pure	II+ I+
Spectrophoto-metry	General High	>1 >18	<50 <10	<0.2 <0.2	<10 <1	NA NA	NA NA	General lab Ultra pure	II+ I+
TOC analysis	General High	>1 >18	<50 <3	<0.2 <0.2	<10 <1	NA NA	NA NA	General lab Ultra pure	II+ I+
Trace metal detection	General High	>5 18.2	<50 <10	<0.2 <0.2	<10 <1	NA NA	NA NA	General lab Ultra pure	II+ I+
Water analysis	General High	>5 >18	<50 <10	<0.2 <0.2	<10 <1	NA NA	NA NA	General lab Ultra pure	II+ I+

*at 25oC
Blue = general lab application; pink= life science applications; green = analytical and chemical applications;
NA = not applicable; ND = not detected; UF = ultrafiltration

6. *Practical considerations for installing a water purification system*

By now you will have a better idea about which level of water purity you need for your experiments; you understand the technologies behind its treatment; and have an appreciation for the sheer variety of applications requiring different types of pure water. However, if you're a lab manager with a decision to make about which type of purification system to install in your lab, there are still a few more things to consider including energy, space and peak usage requirements. In this section we explore some of the more practical factors influencing which water purification system you should select for your lab.

6.1 Design considerations

Probably the most important part of the process after you select the level of water purity you require is to consider the location and use of your lab space. Do you have a single lab? A suite of labs? Or perhaps you are responsible for the whole building's new water purification system?

Space is always at a premium in most labs. Fortunately, there are several space-saving options currently available that minimize the impact on bench footprint (e.g. vertical designs).

There is also a range of supply options, from centralized systems to mains water purified at the point-of-use or even local pretreated water which is subsequently treated further, or 'polished', at the point-of-use. In addition to space, you might want to consider these additional points when selecting the design of your system.

- Is the system to be shared between labs (and therefore cover a broad application range) or will it be restricted to use with a particular application?
- How much pure water will you use, on average, per day?
- What will be the peak times, duration and frequency of use and how will you keep up with demand?
- What about the labwater feed purity? Will this require pretreatment?
- Does the lab require data capture, i.e. validation and tracking, of water purity?
- Is there a need for accessory purification systems such as degassing?
- What other apparatus do you require in addition to the purification system itself? e.g. primary treatment process, reservoirs, 'polishing', storage, dispensing and the purity requirements for each application.

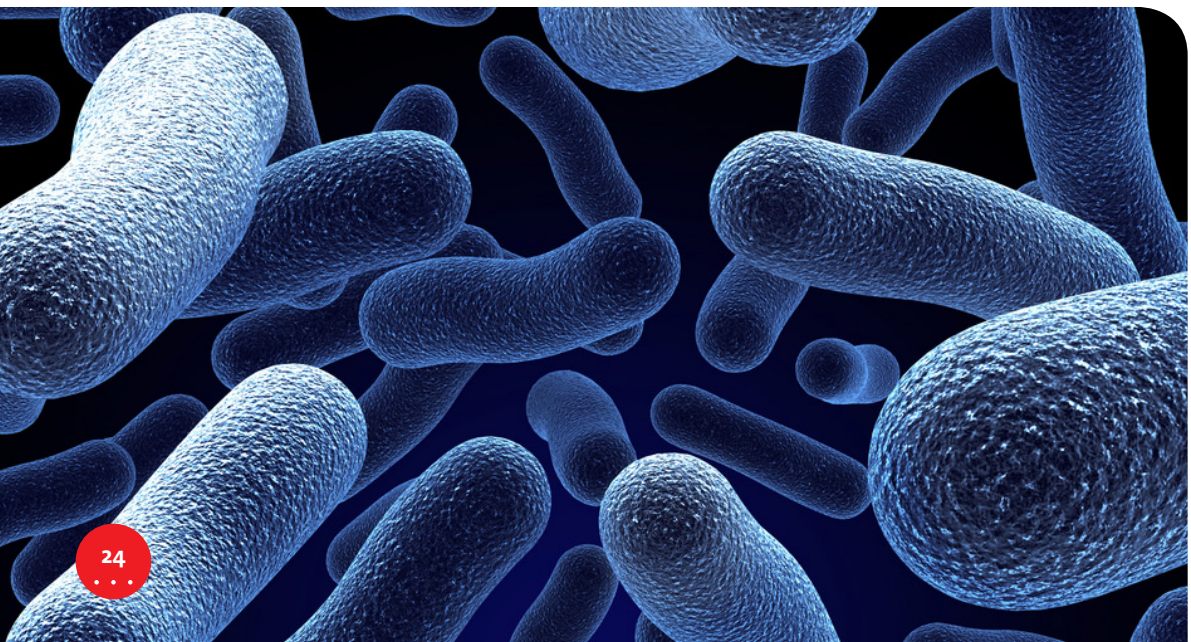
6.2 Water storage

Storage is required in almost all circumstances, usually to ensure there will be an adequate amount available during times of peak usage. Water can be stored in either a recirculating or static system.

In a static system the water in the reservoir is made available after a single pass through a combination of IX resins, UV radiation and filtration. A recirculation storage system makes use of periodic passes through the same treatment process. As you might expect, a recirculating system yields water with comparatively lower levels of contamination than is obtained from static systems as the water is purified on an on-going basis, while ensuring the water is continuously flowing and interfering with biofilm formation.

6.3 Monitoring purity

It's important to be able to be sure that the water you are using is as pure as you think it is. The best way to achieve this is through continuous sampling, ideally in real-time. In-line systems built into the purification are the best option for obtaining the most accurate data. Since ultra pure water is produced in a multi-stage process, there is often the need for multi-stage monitoring in order to ensure a high degree of consistency with regard to purity. By measuring ions and organics (via resistivity and TOC measurements) throughout the purification process, in a truly continuous fashion, you can be certain that your results won't be adversely affected by possible spikes in contaminant levels.



As with all scientific and technological developments, the process of analytical instrumentation and experimental protocol development is an ongoing one. Engineers and scientists continue to push the limits of our ability to detect the most infinitesimal concentrations, thanks to often incredible advances in technology. Sensitivity is rarely an attribute you want to cap when conducting research. As a result, the purity of the water being used in the lab is becoming more important than ever.

7.1 The ongoing optimization of sensitivity

Scientific instrumentation has continued to become increasingly more sensitive in an attempt to enhance both data accuracy and precision. The goal is to improve the signal-to-noise ratio. The first step in optimizing this ratio is to reduce the sources of noise in your samples by ensuring you are using water of high purity, free of interfering contaminants. Here are some examples of new technologies that will be highly dependent on making use of ultra pure water.

- Improved tools and techniques for drug discovery and patient diagnostics.
- Increased sensitivity of already highly sensitive devices, such as those used for HPLC and mass spectrometry.
- New technologies and techniques in areas of molecular biology, e.g. digital and single molecule PCR, microRNA analysis and qPCR, which are highly dependent on the use of apyrogenic, ultra pure water for accurate results.
- The food industry is under higher scrutiny than ever before due to increased public interest. As a result, companies are increasingly making use of advanced analytical methods such as Quadrupole Mass Spectrometry and Electromagnetic Force Vacuum Balance, techniques easily disrupted by trace amounts of contaminants in sample water.
- Nanoparticles are more relevant than ever in ecotoxicological testing since so many of our devices make use of nano-sized materials. With particles such as titanium dioxide inducing mortality in fish at ppb concentrations,[7] detection sensitivity is critical.

7.2 The promise of new water purification technologies

In general, water purification technology has remained relatively unchanged over the past few decades. However, research into this area is on-going, with industrial and academic partners combining to drive the sector forward. For example, researchers at the University of Manchester (UK) have recently been developing a new type of water filter made from graphene.

The graphene filters not only have an exceedingly fine mesh (capable of blocking salts less than nine Angstroms in size) but also allow for an ultra-fast filtration flow due to the graphene working like an "ion sponge", removing a vast amount of ions from the water.[8]

8. *Working with ELGA*

We hope that the information presented here has been of use and that we've managed to demonstrate just how important it is to consider and select the optimum level of water purity for your application.

If you would like to know more on this subject, please contact our team of experts. ELGA has been working in water purification exclusively for almost 80 years now, making us the world leaders in water treatment in the lab.

As an organization, we're committed to ensuring that those working at the bench receive the highest quality of professionalism, and water, possible. Whatever your water needs, get in touch now to see how we can help you make sure all the work you carry out in the laboratory is productive and rewarding.

9. *References*

1. Based on research from the European Commission (DG ENV, June 2009) Final Report, Study on water performance of buildings – http://bit.ly/1iY3e0S; Good Campus, "Energy and Resource Efficiency" – http://bit.ly/10ZaHOZ; University of Oxford, Water Management Strategy Report (January 2011) and AECOM Ltd – http://bit.ly/1m3JEjD

2. ELGA, Pure LabWater Guide – http://bit.ly/1mbVDwm

3. Whitehead, P. Ultra-pure water for HPLC. Why is it needed and how is it produced? Laboratory Solutions, December 1998.

4. ELGA Application Note: Type I ultra pure water crucial for HPLC and UHPLC.

5. Whitehead P. Importance of pure water in modern ion chromatography. Lab Manager Magazine, November 2010.

6. Mostofa KMG *et al.* Dissolved Organic Matter in Natural Waters. Photobiogeochemistry of Organic Matter, in Environmental Science and Engineering. 2013 DOI 10.1007/978-3-642-32223-5_1

7. Bar-Ilan O *et al.* Environmental Science and Technology, 2013 47:4726–43, doi: 10.1021/es304514r.

8. University of Manchester – http://www.manchester.ac.uk/aboutus/news/display/?id=11561

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ELGA Global Operations Centre:

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Web: www.elgalabwater.com