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MORE THAN JUST H_2O – WATER QUALITY IN THE LABORATORY

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How Water Quality Affects Data Quality

Water is unquestionably the lifeblood of the life sciences. Without it, the means to comprehensively study the mechanisms and interactions that make up life on Earth would not exist. The importance of water is rivaled only by its ubiquity in modern research facilities. Today, water is freely available to the vast majority of laboratory scientists at the turn of a tap, flick of a switch, or push of a button. However, not all water is alike. Water is highly susceptible to contamination because it can act as a solvent for an extremely wide range of solutes[1] and sustain life. As such, even within a single laboratory, the water used in one experiment can differ in composition from that used in another – with significant consequences with regard to data accuracy, integrity, and reproducibility.

Water, Too, is a Laboratory Reagent

It is important for anyone using water in the laboratory to regard it as one does any other reagent. The causes for water contamination can broadly be separated into inorganic and organic sources. Inorganic sources include gases and ions dissolved within the water, as well as nonsoluble particulate matter and colloids. Organic sources include metabolic products and synthetic carbon-containing compounds, as well as microorganisms – namely bacteria – and their various waste products and secretions.[1,2]

Types of Inorganic Contaminants

Inorganic contamination affects experimentation in several ways. From a physical standpoint, nondissolved particles can clog micro- and nanometer-sized pathways such as filter pores or microfluidic channels. This property can be harnessed to filter out particulate matter with specifically designed filters,[3] but if clogging occurs outside of filtration scenarios, it can interfere with or even damage very sensitive equipment. From a biochemical standpoint, dissolved inorganic ions are often active agents. Non-alkali metals such as zinc can serve as catalysts for enzyme function, while alkali metals such as sodium and potassium modulate the resistivity and conductance properties of the water while also affecting cellular electrophysiology.[1] Finally, dissolved gases can fall under both categories, forming bubbles to physically alter particle counts or light measurements or changing the solution's pH, thereby modulating biological and chemical behavior.[1]

Types of Organic Contaminants

Organic contamination, usually measured as total organic carbon (TOC),[1] is likewise deleterious to experimental success. It is particularly impactful on chromatographic assays such as HPLC and LC/MS, where it increases background noise and fouls separating columns, resulting in a loss of resolution and sensitivity.[4] Organic compounds can also impair enzyme kinetics, cellular growth, and calcium availability.[1]

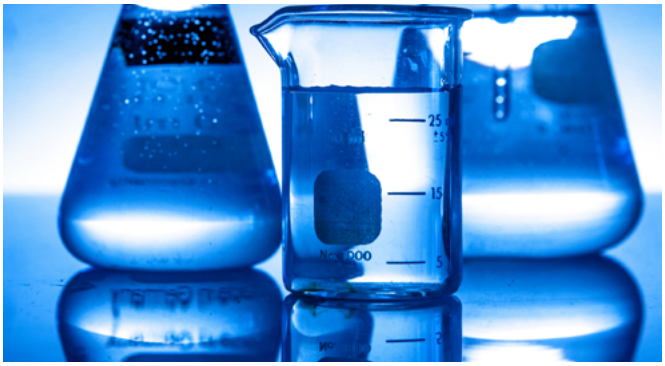
Bacterial contamination is a death knell to the vast majority of experiments. Not only can bacteria exert cytotoxic effects on both cultured and primary cells, they also secrete various agents into water, compromising downstream concentration measurements, interfering with antibody function, and negatively impacting nucleic acid integrity. Attempts to remediate bacterial contamination by killing invading cells should consider that cell death will result in lysis and the spilling of bacterial proteins into the solution. Always consider the downstream requirements before selecting the purification assay used.

The Importance of Monitoring Water

Water quality can be affected in a myriad of ways, and as such, consistent water quality monitoring is vital to ensuring experimental reproducibility within and between laboratories over days, weeks, and years. Successfully undertaking this endeavor requires familiarity with the parameters used to define water quality and the differences between various levels of water purity.

For references, please refer to page 7.

Hidden in the Depths: Determining Water Quality



Since water can become contaminated in so many ways, a wide range of different parameters need to be measured in order to determine water quality. As such, there is no absolute single standard for water quality, and deciding whether water quality is suitable or not depends on circumstance and intended purpose. Several organizations, such as the Clinical and Laboratories Standards Institute (CLSI; formerly the NCCLS, National Committee for Clinical Laboratory Standards) and ASTM International (formerly the American Society for Testing and Materials, ASTM) have released voluntary water quality guidelines, but the ultimate responsibility for implementation and adherence is in the hands of the researcher.

What Parameters are Measured to Assess Water Quality?

In general, overall water quality is determined by assessing ionic purity, organic purity, bacteria levels, and particulate levels. Historically, NCCLS guidelines separated water into three types based on purity levels,, with Type I being the purest (Infographic Table 1). Type I water not only needed to meet more stringent criteria, but also necessitated more measurements, as Type II and III offered no threshold requirements for organic material, particulate matter levels, or pH.[1,2]

The spirit of the NCCLS guidelines have carried over into their successor, the updated CLSI guidelines. These, in lieu of Types I, II, and III, classify water as instrument feed water (IFW), special reagent water (SRW), and clinical laboratory reagent water (CLRW) (Infographic Table 2).[1] The criteria necessary to designate water as CLRW or Type I match closely but the relatively minimal criteria required to define Type II and III water have been dropped altogether. Instead, IFW has no requirements attached to it, while SRW requirements are entirely dependent on the intended application – and may exceed CLRW requirements for certain highly sensitive applications such as HPLC and LC-MS.[1] Broadly, the NCCLS/CLSI guidelines place the onus upon the researcher to know what his or her water quality needs are and act accordingly.

In contrast, ASTM International grades water from Type I (purest) to IV based on clear numerical thresholds. In addition, bacterial contamination is separately evaluated with an additional system (Types A, B, and C) based on bacterial and endotoxin content levels (Infographic Table 3). The ASTM also provides a summary guideline on the minimum requirements for "bio-applications grade" water (Infographic Table 4).

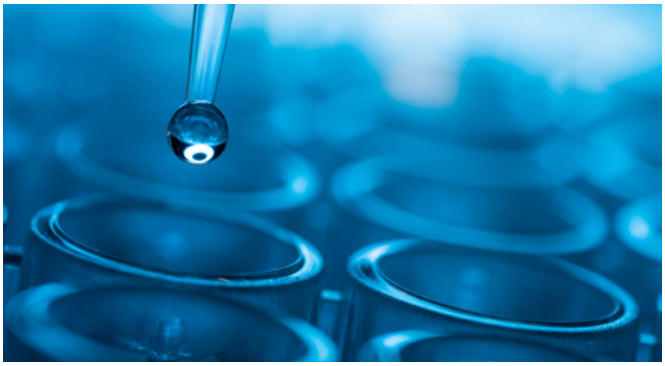
Finally, other organizations have also established water quality guidelines for various applications. The ISO (International Organization for Standardization) and ACS (American Chemical Society) have both set standards for applications involving analysis of inorganic chemicals, while the USP (United States Pharmacopeia) sets standards for water used in the production of pharmaceutical products and injections.[2] While these guidelines may contain useful information, especially for researchers in related fields, it must be noted that they are highly application-specific.

Water Quality Requirements Depend on Researcher Needs

While there is general consensus on the parameters to be measured in order to evaluate water quality, it is important to keep in mind that the actual water quality varies greatly depending on the application that the researcher wishes to perform and the depth of the data that he or she aims to acquire. In most situations, water quality standards are meant to serve as guidelines, offering researchers knowledge regarding how to determine whether their water is a potential source of experimental variability.

For references, please refer to page 7.

Down to the Last Drop: Obtaining Water Quality



After characterizing the composition of existing water, the final step to obtaining laboratory water quality involves carrying out various purification techniques. As previously mentioned, since water quality requirements vary depending on the intended downstream application(s), the exact nature of the purification technique(s) necessary will vary as well. A variety of different protocols and procedures have been devised to remove impurities from water, with each specific technique generally specialized for the removal of one particular type of impurity. As such, a combination of multiple methods is usually necessary to obtain water of sufficient quality for experimental needs.[1]

Basic Water Purification Techniques

Distillation, where water is boiled and the resulting steam collected via condensation, is perhaps the simplest water purification technique. Distillation can remove a host of impurities, including microorganisms, ions, most organic products, and dissolved gases.[2] However, distillation is logistically intensive – requiring large amounts of storage space, time, heat energy, and maintenance – and does not remove chemicals such as toluene, benzene, or chlorine that have boiling points lower than the boiling point of water.[2]

Filtration is also a relatively simple technique where water is passed through porous filters in order to separate objects larger than the pore size. These filters are typically constructed using activated carbon, cellulose, or synthetic polymers, and are classified as depth/particle, micro-, or ultrafilters based on pore size, which ranges from 10-25 µm down to 0.01-0.1 µm.[1-3] Sequential filtration is highly effective in removing particulate matter, with ultrafiltration also capable of removing endotoxins, pyrogens, and enzymes.[1] Because of this, ultrafilter pore sizes are often expressed as molecular weight cut-offs (MWCOs).[2]

Removing Ions

In general, filtration does not excel at removing ions from water, and filtered water is usually subjected to deionization processes. Reverse osmosis (RO), like filtration, uses a filter to separate unwanted elements from water. However, in RO, water is forced through a semi-permeable membrane (MWCO: 100-200 Da)[2] using hydraulic pressure in order to overcome the opposing osmotic pressure generated by the concentration gradient across the membrane. The end result is that solutes are retained on the pressurized side while the solvent – in this case, water – passes through. RO is effective in removing 90-99% of particles, ions, organics, and microorganisms in a single step,[2] but is hampered by membrane fouling and slow flow rates.

Ion-exchange and electrodeionization (EDI) can also be used to deionize water. In both techniques, ions are extracted using ion-exchange resins. Direct ion-exchange swaps unwanted ions for H^+ and OH^- ions, the components of water, while EDI applies a current to pull ions through semi-permeable exchange membranes to an anode or cathode depending on polarity.[2] Ion-exchange resins will become exhausted over time, but EDI overcomes this problem by using a low electric current to continuously regenerate resins.[4] Deionization techniques will not remove any other contaminants from the water, and deionization equipment can easily become contaminated if the water is not pretreated prior to deionization.[1]

Targeting Organisms and Organics

Finally, irradiation with UV light at 185 and 254 nm breaks bonds between carbon, nitrogen, and hydrogen atoms. This not only eliminates living microorganisms and denatures contaminants such as enzymes and nucleic acids, but also breaks up organic species. UV light at 185 nm in particular ionizes organic species through the generation of free radicals, allowing for their removal during subsequent deionization steps.[2]

Knowledge is Power When It Comes to Water Purification

Generating pure laboratory water requires multiple steps and processes. Fortunately for researchers, technological advances now allow these purification steps to be sequentially and automatically performed by a single instrument, allowing pure water to be accessed at the push of a button. Nonetheless, it is important for researchers to have a firm understanding of the different water contaminant types, their potential experimental impact, and the techniques used to remove them, as laboratory water systems come in different shapes, sizes, and capabilities. Having the knowledge of how pure water is generated gives the researcher the power to ensure that his or her experiments are being performed with water that meets their requirements.

For references, please refer to page 7.

WHAT'S IN YOUR WATER?

MICROORGANISMS

- Microorganism contamination is usually bacterial and heavily impacts downstream assays involving cells or cellular material
- Directly leads to death in most cultured cells; can induce phenotypic changes in survivors
- Secrete nucleases, pyrogens, and phosphatases, affecting nucleic acid and protein integrity
- Confounds cell counts and protein/nucleic acid concentration measurements
- Interferes with downstream assays (e.g., PCR, western blot, ELISA) by destroying source material quality or impeding probing mechanisms (e.g., antibody binding specificity)

REMOVAL

- Microorganisms can be killed using UV light irradiation at 185 or 254 nm
- Dead microorganisms may leave cellular debris/internal cellular contents in solution
- Filtration can remove both microorganisms and cellular debris
- Equipment maintenance is critical as microorganisms can foul filters and form biofilms

IONS

NON-ALKALI METALS

- Metals such as Fe, Cu, Zn, and Mn are involved in many physiological processes, serving as catalysts, enzyme inhibitors, or chelators
- Heavy metals (e.g., Hg, Zn, Pb) are cytotoxic

ALKALI METALS

- Na^+ and K^+ ions are critical to electrophysiology and affect water conductivity and resistance
- Mg levels are important to PCR efficiency

REMOVAL

- Ion-exchange resins swap unwanted ions for H^+ or OH^- ions
- Resins combined with electric currents pull ions out of solution towards an anode or cathode depending on charge polarity
- Deionized water is "hungry" – highly susceptible to ionic contamination leached from storage containers
- Ion content is measured by resistivity, with 18.2 MΩ·cm at 25 °C the industry standard for pure deionized water

PARTICULATE MATTER & COLLOIDS

- Nonsoluble particulate matter can be present in the form of minerals, sediment, sand, and other similar materials
- Can clog filter pores and small channels, such as those commonly used in microfluidic or chromatography instruments
- Particulate matter can directly damage sensitive equipment used in biological assays; clogs increase pressure levels within instrument systems, indirectly damaging pumps, injectors, and sensors
- Colloids modulate water viscosity, potentially affecting system pressures and causing clogs

REMOVAL

- Microfiltration with a 0.22 µm pore size is sufficient to remove most particulate matter; this is the minimum requirement for clinical laboratory reagent-grade water under CLSI specifications

GASES

- Gases are absorbed and dissolved into water via atmospheric contact
- Excess N_2 and O_2 may confound experimental measurement accuracy, particularly in the ecological sciences
- Cl_2, CO_2, NH_3 can ionize, altering water pH
- All gases may precipitate and form bubbles, affecting spectrophotometric measurements and blocking microfluidic channels

REMOVAL

- Distillation partially removes dissolved gases, but a new equilibrium will be established upon atmospheric contact
- Specific gases can be removed by subjecting the water to chemical reactions intended to consume the target gas
- The products of ionizing gases can be removed using ion-exchange or electrodeionization (EDI); continued application will eventually exhaust gas reserves

ORGANIC CONTENT

- Organics are defined as compounds with carbon as a principle constituent
- This can include decayed plant matter, solvents (e.g., toluene, benzene), and byproducts of combustion
- Organic contamination is detectable by chromatographic assays (e.g., HPLC, LC/MS), leading to increased noise or confounding peaks; organic compounds can also generate background fluorescence
- Hydrocarbons are cytotoxic, impairing cellular function and enzyme activity

REMOVAL

- UV irradiation breaks carbon-hydrogen bonds, destroying organic molecules
- Reverse osmosis is excellent for removing organic compounds due to the low MWCO point

1 NCCLS GUIDELINES FOR CLASSIFICATION OF WATER TYPES (1998)

CONTAMINANT (PARAMETER)	TYPE III	TYPE II	TYPE I
Ions (Resistivity; MΩ·cm @ 25 °C)	0.1	1.0	1.0
Organic Materials (TOC ppb)	NS	NS	Carbon filtration
pH	NS	NS	5-8
Particulates >0.22 µm (Units/ml)	NS	NS	0.22 µm filtration
Colloids (Silica; mg/L)	1.0	0.1	0.05
Bacteria (CFU/ml)	NS	<1000	<10

2 CLSI SPECIFICATION FOR REAGENT LABORATORY WATER (2006)

WATER TYPE	SPECIFICATIONS
Clinical laboratory reagent water (CLRW)	Microbial content <10 CFU/ml >10 MΩ·cm @ 25 °C Free of particulates >0.22 µm TOC <500 ppb
Special reagent water (SRW)	Application defined
Instrument feed water (IFW)	NS

3 ASTM REAGENT GRADE WATER SPECIFICATIONS (GUIDELINE D1193-06-2011)

CONTAMINANT (PARAMETER)	TYPE IV	TYPE III	TYPE II	TYPE I
Ions (Resistivity; MΩ·cm @ 25 °C)	0.2	4.0	1.0	18
Organic Materials (TOC ppb)	NS	200	50	50
pH	5-8	NS	NS	NS
Chloride (µg/L)	<50	<10	<5	<1
Sodium (µg/L)	<50	<10	<5	<1
Colloids (Silica; mg/L)	NS	500	3	3

CONTAMINANT (PARAMETER)	TYPE A	TYPE B	TYPE C
Endotoxin (EU/ml)	<0.03	0.25	NS
Bacteria (CFU/ml)	1	10	1000

NS: not specified

4 ASTM STANDARD GUIDE FOR BIO-APPLICATIONS GRADE WATER (GUIDELINE D5196-06-2006)

PARAMETER	STANDARD
Ions (Resistivity; MΩ·cm @ 25 °C)	18
pH	NS
Organic Materials (TOC µg/L)	20
Chloride (µg/L)	NS
Sodium (µg/L)	NS
Total Silica (µg/L)	NS
Bacteria (CFU/ml)	100
Endotoxin (EU/ml)	0.01 (or as required)
Nucleases and Proteases	As required

Article 1 - How Water Quality Affects Data Quality

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1. R. Nabulsi and M.A. Al-Abbadi. "Review of the impact of water quality on reliable laboratory testing and correlation with purification techniques," *Lab Med.* 45(4):e159-165, 2014.
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Article 2 - Determining Water Quality

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Article 3 - Down to the Last Drop – Obtaining Water Quality

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1. National Institutes of Health (NIH). "Laboratory Water: Its importance and Application," 1-22, 2013. Available at: http://orf.od.nih.gov/PoliciesAndGuidelines/Documents/DTR%20White%20Papers/Laboratory%20Water-Its%20Importance%20and%20Application-March-2013_508.pdf, accessed Sept 26, 2018.
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