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Review Article

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Decoding the Quality of Water in the Clinical Laboratory

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Abstract

Water is the most extensively utilized reagent in clinical laboratories, fundamental to reagent preparation, sample dilution, and instrument maintenance. Its purity directly influences the accuracy, reliability, and reproducibility of diagnostic results. This review comprehensively discusses the principal parameters defining water purity-conductivity, resistivity, total organic carbon (TOC), microbial load, and particulate content-and examines how global standards by CLSI, ISO, and ASTM govern these specifications. Laboratory water is categorized into Type I, II, and III (or Grade equivalents by ISO), each suited for distinct applications based on resistivity, TOC levels, and microbial thresholds. The article details the requirements for each type, emphasizing the necessity of ultrapure Type I water for high-precision assays like HPLC and mass spectrometry, while Type II and III serve routine analyses and equipment feed needs. Various purification methods-including distillation, ion exchange, reverse osmosis, ultrafiltration, and ultraviolet oxidation-are explored, highlighting their mechanisms, advantages, and limitations. The importance of robust monitoring systems to assess resistivity, TOC, and microbial content is underscored, ensuring water quality aligns with analytical demands and prevents instrument damage or assay interference. Adherence to stringent water quality standards not only sustains laboratory accreditation but also safeguards public health by upholding the integrity of diagnostic outcomes.

Keywords: ***Water purity, reagent grade water, CLSI, laboratory standards, water purification.***

Introduction

There are numerous uses of water in a clinical laboratory like preparation of reagents, as a diluent for calibrators and controls, in hot water baths, and to wash and rinse glassware. It is perhaps the most commonly used reagent in the clinical laboratory. But while working in a clinical laboratory, one should be very careful about the quality of water used to reduce bacterial contamination or to lessen the risk of damaged equipment, contamination of reagents or compromised experimental or diagnostic results. For this purpose, biomedical research workers should become familiar with and apply the appropriate water grade most suited to their needs [1]. Regular monitoring of the water used in clinical laboratory assays is essential to ensure accurate results. Otherwise, improper water quality can lead to inaccurate results and contamination, thus affecting the public health.

Common contaminants of water

Water is known as the universal solvent. The polarity of the molecule and the unique hydrogen bonds account for its ability to react with neutral organic molecules and establish hydrogen bonding with others. For this reason, water is easily contaminated by chemical solids, gases, vapours and other particulate matter [2]. The common contaminants of water are enlisted below:

- Sodium and silica from glass, plasticizers
- Ions that leach from conduit lines
- Microbial species and their endotoxins
- Soluble organic contaminants from deionizer resins
- Cations such as sodium, calcium, magnesium or iron
- Anions such as bicarbonate, chloride and sulfate

Apart from those listed above, there are numerous others found in tap water, many of which may interfere with lab workflows demanding high precision, sensitivity and instrument maintenance.

Measure of contaminants in water/Definitions of Water Purity [3]

There are several parameters that define the purity of water, which are used to describe types of pure water. Let us first define the parameters of water purity.

- Conductivity measures the ease with which a water sample conducts electricity. Electricity is conducted through the ions in water. So, the more ions present in the water, the higher the conductivity. This is measured by a conductivity meter and should ideally be taken online. The unit of measurement is the Siemen(S), microsiemens/centimeter (µS/cm) or micromho/cm.

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Conductivity increases with temperature. Therefore, the values are to be adjusted at 25 °C.

- Resistivity or Specific resistance measures how difficult it is for an electric current to travel through a water sample. It is the tendency to resist conducting electricity. Resistivity is the inverse of conductivity. The electrical resistance is measured between the opposite faces of a 1-cm cube of an aqueous solution at a specified temperature. The higher the resistance, the less the ionic content of water and higher will be the conductivity. The converse is also true. The unit of measure is mega ohm centimetre (MΩ-cm). It varies with temperature. The maximum values possible are 18.2 to 18.3 MΩ-cm at 25°C.
- Total organic carbon (TOC): The ammount of organic compounds in a water sample is quantified by the total organic carbon content. The unit of measure is parts per million (ppm) or parts per billion (ppb). This organic content may either be contaminants or nutrients for microorganisms. This is usually measured by a process that oxidises the organic compounds present in the water sample and then quantifies the oxidation products generated. They then measure either the carbon-di-oxide or the acid by product or the change in conductivity. Feedwater systems for central arrangements have a TOC levels in the range of 200-500 ppb. The best high purity water (usually used in HPLC) should be in the 1-5 ppb range. Measurement of TOC of centralized feedwater systems are conducted off line quarterly but when the required TOC is very low (e.g. <50ppb), online measurements are advised.
- Microbiological Content: The microbiological content of viable organisms, is determined by total colony count after incubation at 36 ±1°C for 14 hours, followed by 48 hours at 25±1°C, and reported as colony-forming units per mL (cfu/mL).
- Particulate Matter: When water is passed through a membrane filter with a mean pore size of 0.2 µm, it is considered to be free of particulate matter; when water is passed through a bed of activated carbon, it is considered to contain minimum organic material.

Lab Water Grade water

The reagents and chemicals used in a clinical laboratory are considered to be of reagent grade. Water, as explained is used widely in the clinical lab and therefore the purity of the water used is of paramount importance. The Clinical and Laboratory Standards (CLSI), formerly known as NCCLS and other regulatory bodies like ASTM (American Society for Testing and Materials) and ISO (International Organization for Standardization) have laid down the specifications for the characteristics of water to be used in the clinical lab. Formerly, “Type I” was used to designate ultrapure waters, and Type II and Type III were used for inferior qualities of water. (vide Table 1)

Type I water is the purest form of water and must meet the following requirements:

- Resistivity greater than 18 MΩ.cm
- Conductivity less than 0.056 µS/cm
- Total organic carbons less than 50 ppb
- Used for HPLC mobile phase preparations, blank preparations, tissue culturing, mass spectrometry, determination of trace metals, enzyme, and electrolyte measurements, sample dilution in GC-MS, reagents for molecular biology, preparation of calibrators reference materials and other processes requiring the highest levels of purity, maximal precision and accuracy.

This type of water should be used immediately after production because of the difficultly in maintaining the high resistivity while drawing off the water and storing. So, there are no specifications for storage of type I water.

Type II water is pure enough for specialised use but not considered ultrapure. The specifications are

- Resistivity greater than 1 MΩ.cm
- Conductivity less than 1 µS/cm
- Total organic carbons less than 50 ppb
- Used for clinical analyzers, instrument feeds, electrochemistry, dilution of samples, buffer preparation, pH solution preparation, microbiological media preparation, feed for analyzers, washing machines, autoclaves and to create Type I water. Only minimum quantities should be stored and steps to be taken to reduce chemical and bacterial contamination.

Type III water is produced from normal tap water by the process of reverse osmosis [1,2]. The properties are:

- Resistivity greater than 4 MΩ.cm
- Conductivity less than 0.25 µS/cm
- Total organic carbons less than 200 ppb
- Used for rinsing glassware, general and noncritical applications, hot water baths, washing machine feeds, autoclaves and certain qualitative procedures like urine analysis.

The ISO utilizes the term “Grade” in instead of type, with significant variation of criteria. The scope of the ISO standard is limited to laboratory reagent water for analysis of inorganic chemicals.

The CLSI uses the terms CLRW (Clinical Lab Reagent Water) and SRW (Special Reagent Water) and Instrument Feed Water to specify the different grades of pure water [1]. This clinical grade laboratory water is typically used for washing cuvettes (in order to remove carry over) washing of sample probes and pipetting syringes and reagent probes. These are necessary to eliminate cross-contamination across samples and reagents. This water is also used in incubator baths to achieve accurate photometric measurements. The CLRW specifications include ions, particulates, organic matter and microbial load. The criteria outlined by the CLSI are described below.

The resistivity specification restricts the ionic impurity concentrations and requires the elimination of carbon di oxide. This is adequate for general chemical, electrolyte assays enzyme immunoassays, lipid and protein assays, but determination of trace elements requires higher resistivity at 18.2 MΩ.cm. Low level of particulates is an absolute requirement as particles can clog needles and tubings and may even induce biofilm formation which affects the transmissivity and path length of spectroscopic cells.

The ASTM establishes specifications for Types I, II, III, and IV reagent grade water and is further classified as Type A, Type B,

or Type C depending on the applicable bacteriological and endotoxin quality.

For all practical purposes, the CLSI guidelines are followed minutely. There are additional specifications for the desired quality of water for specific biopharmaceutical applications like water for injection, sterile water and USP purified water as laid down in US pharmacopeia. USP purified water (which includes conductivity, TOC, and microbial parameters) may be used for instrument feed systems, but USP waters (including water for injection) are not permitted for many reagent lab water end use applications.

Table 1: Reagent grade water specifications as laid down by NCCLS

Parameter	Type I	Type II	Type III
Bacterial load (cfu/ml)	10	1000	Not specified
Resistivity	10	1.0	0.1
Silica max. (mg/l)	0.05	0.1	1.0
Particles	0.22micron filtration	Not specified	Not specified
Organics	Carbon filtration	Not specified	Not specified

Table 2: Clinical Lab Standard Institute (formerly NCCLS), Reagent Laboratory Watter (similar to Type I water)

Parameter	Clinical Laboratory Reagent Water CLRW
Microbial, max. (CFU/ml), plate count	10
pH Units	NS
Resistivity, min. (megaohm) at 25°C	10
Silica	NS
Particles and Colloids	0.22micrometers filter or smaller (Water passed through 0.2-µm filter)
Organics (TOC), (parts per billion)	500 (Water passed through activated carbon)

Table 3: American Society for Testing and Materials Reagent Grade Water Specifications

Parameter	Type I	Type II	Type III	Type IV
Resistivity min. MΩ cm (25°C)	18	1	4	0.2
pH, units(25°C)	NA	NA	NA	5-8
TOC, max (µg/l)	50	50	200	NS
Sodium, max (µg/l)	1	5	10	50
Chloride max (µg/l)	1	5	10	50
Total silica max (µg/l)	3	3	500	NA
	Type A	Type B	Type C	
Bacteria, max CFU/ 100ml	1	10	1000	
Endotoxin EU/ml	<0.03	0.25	NA	

Methods of Water Purification

The different methods of water purification are briefly outlined below.

i. Distillation - One of the oldest methods, distillation includes boiling the water and then condensing it. It is used to separate a volatile substance from less volatile substances. The disadvantage of using distillation for preparation of reagents include the carryover of volatile impurities into the distillate. Further, since the process is time-consuming, distilled water must be stored during which it runs a high risk of contamination by leaching of minerals from the container into the distillate. Entrapped water droplets may pass on contaminants like sodium, potassium, manganese, carbonates, and sulphates. Distillation is capable of removing a wide range of contaminants. But, monitoring of the process is essential to ensure water quality and the process is labour intensive. For all these reasons, distilled water does not meet the conductivity requirement of Type I water [3].

ii. Filtration is a common process performed in households as well as laboratories and involves the removal of particulate matter with the help of filters. In descending order of particle capture efficiency, the different filtration processes may be enlisted as reverse osmosis, nanofiltration, ultrafiltration, microfiltration and particle filtration. The pore size of the filters is to be designed according to the filter's efficiency (Beta Ratio) at that flow /flux of water. They may range from 10 or 25 microns in pretreatment stages to 0.45µm to 0.2µm absolute at the point of dispense. Filtration is used usually prior to any other purification processes.

iii. Ion - Exchange or Deionization (DI) is perhaps the only technology that produces Type 1/Ultrapure RGW.As the name implies, the process involves removal of ions to yield de-ionized water free of minerals. The process includes passing feed water through columns of insoluble resin polymers which extract H+ and OH- ions in exchange for the ionic impurities in the water. For this purpose, mixing together cation and anion resins is essential to ensure complete deionization of water. These DI columns have a finite ion binding capacity and hence a limited shelf life and are unable to remove bacteria, pyrogens and organic matter. Ion exchange beds are prone to microbial growth. So DI systems should undergo proper pre-treatment and maintenance. But this water gets contaminated easily and causes corrosion.

Electrodeionization (EDI) combines electro-dialysis and ion exchange technology and is continuously regenerated by the electric current running through the system. This

method is obviously more efficient but does not remove organics and particulate matter.

iv. Reverse osmosis (RO) is a process in which water is passed through a semi-permeable membrane that removes 95 to 99% of organic compounds, and bacteria but dissolved gases. A storage tank is needed to collect and distribute the purified water as the process takes some time.RO requires some pretreatment of the impure water to prevent damage to the membrane. It is an excellent treatment process and when combined with adsorption(with activated carbon) and UV oxidations system can provide water suitable for varied applications.

v. Ultrafiltration methods are used to eliminate bacterial endotoxins and nucleases that may interfere with cell culture preparations but cannot remove dissolved material.

vi. Ultraviolet oxidation (Photochemical oxidation) at 254 nanometers can remove many microbial offenders and breakdown many ionic organic compounds at 185nm which are then removed by de-ionization. But the process does not remove ions, colloids, or particulates [3,4].

Monitoring Water Purity

Dissolved salts and inorganics are monitored on-line in laboratory water systems. pH is not an effective measurement to determine water purity as the low conductance of water affects the stability of pH meters. Instead, special sensors are used for parameters like pH, dissolved oxygen, turbidity etc. Resistivity and conductivity are measured online with compensation for temperature. The need for monitoring TOC is to anticipate the maintenance of the instrument. Bacterial contamination is measured by plating a sterile 0.22 µm membrane, incubating for 3 to 5 days and counting the colony forming unit. These monitoring strategies are essential to prevent costly repairs of instrument and potential downtime issues. All of these contribute to the reliability and credibility of the laboratory.

Conclusion

Water is the most ubiquitous yet often underestimated reagent in the clinical laboratory, with its purity directly impacting the reliability of analytical results and the longevity of laboratory instruments. The myriad contaminants-whether ionic, organic, microbial, or particulate-pose significant risks to both patient outcomes and laboratory operations if left unchecked. It is therefore imperative that laboratory professionals not only understand the various grades and standards of water quality, such as those outlined by CLSI, ASTM, and ISO, but also implement rigorous purification, monitoring, and maintenance protocols tailored to their specific analytical needs. By employing appropriate purification technologies-ranging from distillation and reverse osmosis to advanced methods like electro-deionization and ultraviolet oxidation-and by adhering to strict monitoring practices for parameters like resistivity, TOC, and microbial load, laboratories can uphold the high standards required for accurate diagnostics and research. Ultimately, ensuring optimal water quality is not merely a regulatory requirement; it is a fundamental component of delivering precise, credible, and safe laboratory services that safeguard public health.

List of Abbreviations

CLSI: Clinical and Laboratory Standards Institute
NCCLS: National Committee for Clinical Laboratory Standards
ASTM: American Society for Testing and Materials
ISO: International Organization for Standardization
TOC: Total organic carbon

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