A GUIDE FOR LABORATORIES

Pure lab water guide

An essential overview of lab water purification applications, monitoring and standards.

Dedicated to Discovery
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The pure labwater guide

Introduction

The Pure LabWater Guide is an essential resource for individuals who use pure water or wish to learn more about the subject. Providing an overview of water purification requirements, techniques and applications in science and medicine, this educational guide will enable you to choose the correct grade of water and most reliable method of production at an economical cost to both your budget and the environment.

Challenges: Impurities and Variations in Drinking Water

Water for most laboratory and clinical applications is usually purified from drinking water. However, the unique ability of water to dissolve (to some extent) virtually every chemical compound and support practically every form of life means that drinking water supplies contain many substances in solution or suspension; additional impurities are derived during the drinking water purification process. Furthermore, unlike other raw materials, drinking water may vary significantly in purity both from one geographical region to another and from season to season.

In today’s laboratories, the availability of pure water is essential, and while domestic consumers consider tap water to be “pure”, laboratory scientists and healthcare professionals regard it as highly contaminated. Analytical and experimental scientists are concerned with elements and compounds at concentrations in the parts per billion (ppb) range or lower as many of these contaminants can have a negative effect on applications through their interaction with other substances, including the substance under analysis.

There are 5 classes of impurities found in natural and drinking water:

- Suspended particles
- Dissolved inorganic compounds
- Dissolved organic compounds
- Microorganisms & biomolecules
- Dissolved gases

The overall objective of water purification methods for scientific and medical applications is to remove drinking water impurities while minimising additional contamination from purification system components and bacterial growth.
“Pure water is the most common substance that underpins a vast number of diverse scientific and medical applications – its importance should never be undervalued.”

**How to Use This Guide**

This guide is written by ELGA and is based on more than 70 years’ experience dedicated solely to the research, design, manufacture and installation of water purification systems. The comprehensive Pure LabWater Guide is an amalgamation of our original Pure LabWater Guide and Pure Clinical LabWater Guide, first published in 1991 and 2003 respectively. In addition to providing updates in the field of water purification (i.e. new water purification technologies, additional applications and revised standards) the guide has been designed so that the information you require can be more easily accessed. Throughout this guide you will see hints and tips and “Pure Facts” about water purification with diagrams that summarise important technologies, systems and processes. A glossary is provided at the back so that you can simultaneously refer to and understand technical terms you are less familiar with.

This guide is divided into 4 easy-to-access sections.

- Research and testing (section 1)
- Clinical diagnostics (section 2)
- Healthcare (section 3)
- Water purification overview (section 4, further divided into 5 subsections)
  - Production of drinking water
  - Impurities in drinking water
  - Water purification technologies
  - Maintaining the purity of purified water
  - Purified water standards

**Section 1
Research and Testing**

Focuses on the vast range of applications that are performed in different laboratories, spanning basic glassware washing and rinsing through to the most critical molecular biology and cell culture techniques. It outlines the types of purified water required for each category of application.

**Section 2
Clinical Diagnostics**

Highlights the importance of using extremely pure water to obtain valid and reliable chemical test results. It outlines the international standards and regulations required for these applications.

**Section 3
Healthcare**

We outline numerous applications in Healthcare that require high-purity water, including the decontamination cleaning process for rinsing surgical instruments (e.g. endoscopes) and the production of steam for instrument sterilisation. It details the stringent guidelines and purified water standards that are now being imposed for these applications.

**Section 4
Water Purification Overview**

Provides a comprehensive overview about pure water, detailing the types of impurities found in water and the technologies, system design and components that are required to successfully remove them. The selection of the initial stages of a purification system will depend on the characteristics of the feedwater and the entire process starts with a pretreatment stage. The major water purification technologies are outlined and each has its advantages and restrictions; for example, some technologies can remove large amounts of several impurities, while others can remove one specific type of impurity down to extremely low levels.

There are a myriad of different published standards that define the water quality required for specific applications. ASTM. (American Society for Testing and Materials) and ISO. (International Organization for Standardization) 3696 provide guidelines for laboratory applications; CLSI. (Clinical and Laboratory Standards Institute) guidelines define water quality requirements for clinical laboratories. Some laboratories will also adopt standards outlined in the European, US or Japanese Pharmacopoeia. However, very few of these standards are specific to your particular application; going too far will result in unnecessary costs or not far enough will endanger the accuracy of your results. This guide will allow you to navigate through the maze of standards and help you to choose with ease the right type of purified water and method of production to provide you with the correct purity at an economical cost to your budget and the environment.
ABOUT ELGA

As an integral part of Veolia, the world’s leading water service company, ELGA provides a reliable source of water that economically meets the required compliancy of all our customers’ scientific and medical applications. With more than 70 years’ experience dedicated solely to pioneering water purification systems, we are continuing to apply cutting-edge research with innovative and ergonomic design. ELGA delivers robust and easy-to-install systems to meet our customers ever changing needs. We also work very closely with leading laboratory instrument companies to customize water purification systems for specific applications. Additionally, we play a pro-active role with the water standards organisations which develop and recommend the Lab water quality requirements.

With a network of over 600 service centres worldwide, ELGA guarantees an unrivalled package of service and support, no matter where you are, for its entire range of water purification systems.

Walter Lorch founded ELGA.
Distillation was at the forefront of water purification, however the limitations of this technology, with regards purity, provided a driver for change. The cartridge-type deioniser was invented by Walter Lorch

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1960 - 1970
ELGA collaborated with London School of Pharmacy to develop products aimed at the hospital market, laboratories and general industry

1980 - 1989
ELGA established the School of Water Sciences. Walter Lorch published ‘The Handbook of Water Purification’. ELGA was the first to introduce UV photo-oxidation in a water purification ‘system’ that provides high-purity water at minimum costs. ELGA wins the Queen’s award for design. ELGA invented the “Type II” or distillation replacement system, which became incorporated into their ‘Option’ range of products. ELGA developed MEDICA, the first water purification systems specifically designed for the clinical diagnostic market. ELGA launch the PureSure system (using multistage monitoring) as well as our real-time method of TOC monitoring

1990 - 1999
ELGA launched the PURELAB UHQ, a combination of ion exchange, membrane processes, adsorption and photo-oxidation in a water purification ‘system’ that provides high-purity water at minimum costs. ELGA became the Laboratory Water division of Veolia. ELGA launched the Option-E5, the first laboratory purification system to feature recirculating Electro Deionisation of treated water

2000
ELGA launched BIOCURE the first product specifically designed to meet the latest stringent water standards in medical applications

2003
ELGA launched the CENTRA systems, the first packaged centralised system for laboratory water purification

2004
ELGA launched BIOPURE the first product specifically designed to meet the latest stringent water standards in medical applications

PURE FACTS – WATER DEFINITIONS

Numerous water quality standards exist worldwide defined in laws and regulations, in addition to generic classifications of water adopted by companies based on physical and chemical limits. There is often some confusion about water quality definitions, so let’s revisit some basics:

- **Purified water** is a general term meaning that water has been mechanically filtered or processed to remove impurities.
- **Demineralized water** refers to water that has undergone a process to remove minerals and salts from it. Unlike distillation, demineralization does not remove bacteria or viruses.
- **Deionized water** (DI water, DIW or de-ionized water), often synonymous with demineralized water / DM water, has almost all of its mineral ions such as sodium, calcium, iron, copper, chloride and sulfate removed. Deionization is a chemical process that uses ion-exchange resins to exchange hydrogen and hydroxide ions for dissolved minerals and then recombine to form water. Most non-particulate water impurities are dissolved salts, so deionization produces highly pure water that is generally similar to distilled water.
- **Distilled water** has been boiled into vapor and condensed back into liquid in a separate container. Impurities with a higher boiling point than that of water remain in the original container. Double-distilled water (abbreviated “ddH2O”, “Bidest. water” or “DDW”) is prepared by slow boiling the uncontaminated condensed water vapor from a prior slow boiling.

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Section 1

Research and analysis applications

Scientists perform a vast range of applications in many different kinds of laboratories. Water has a set of unique properties as a solvent (such as a high dielectric constant and good solubility for minerals) and is therefore often used in analytical applications. Therefore, different grades of water must be purified and utilised to match the required procedures or appliances. Water is one of the major components in many applications, but the significance of its purity is often not recognised.
**ABOUT THIS SECTION**

In this section we highlight some common applications and provide guidance on the water quality required. We also provide some guidance on what purification technologies you should be looking for in your water purification system.

There are many water quality standards published throughout the world, however only a few are relevant to specific research applications. This has resulted in the majority of water purification companies, including ELGA, adopting broad generic classifications defined by measurable physical and chemical limits. Throughout this guide we will refer to the “Types” of purified water referred to in this chart (see below).

<table>
<thead>
<tr>
<th>Grade of water</th>
<th>Resistivity MΩ-cm</th>
<th>TOC (PPB)</th>
<th>Bacteria</th>
<th>Endotoxin (EU/mL)</th>
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<td>&lt;5</td>
<td>&lt;1</td>
<td>&lt;0.03</td>
</tr>
<tr>
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<td>&lt;1</td>
<td>&lt;0.03</td>
</tr>
<tr>
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<td>&lt;50</td>
<td>&lt;10</td>
<td>n/a</td>
</tr>
<tr>
<td>Type II</td>
<td>&gt;1</td>
<td>&lt;50</td>
<td>&lt;100</td>
<td>n/a</td>
</tr>
<tr>
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<td>&gt;0.05</td>
<td>&lt;200</td>
<td>&lt;1000</td>
<td>n/a</td>
</tr>
</tbody>
</table>

Table 1: Details of various water grades and typical applications. TOC, total organic carbon; ppb, parts per billion; CFU, colony forming units; EU, endotoxin units.

**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; such as GC (Gas Chromatography), AAS (Atomic Absorption Spectrophotometry) and ICP-MS (Inductively Coupled Plasma Mass Spectrometry). Type I is also required for molecular and microbiology applications as well as mammalian cell culture and IVF (In Vitro Fertilisation).

**TYPE II+**

**TYPE II**

The grade for general laboratory applications requiring higher inorganic purity.

**TYPE III**

The grade for general laboratory applications. This may include media preparation, pH solutions and buffers and for certain clinical analysers. It is also common for Type II systems to be used as a feed to a Type I system*. These systems can also be used to feed Type I systems*

*The production of ultra pure water (18.2 MΩ-cm resistivity, <5 ppb TOC) from tap water is usually carried out in two stages – pretreatment and polishing. Ideally, pretreatment reduces all the major types of impurities – inorganic, organic, microbiological and particulate – by over 95%. This can be most effectively achieved using reverse osmosis or reverse osmosis combined with ion exchange or EDI. Alternatively ion exchange can be used but this cannot reduce the levels of organic, bacterial and particulate impurities to the same extent. The better the pretreatment the higher potential quality of the final ultra pure water.*
Analytical and general applications of purified water

**ELECTRO-CHEMISTRY**

Since these techniques rely on the sensitive measurement of tiny electrical signals, it is vital that the water used produces minimal interference due to background contamination. ASMT Type II water, typically with a TOC (Total Organic Carbon) <50 ppb and a bacterial count below 1 CFU/ml (Colony Forming Units per millilitre) is recommended for electrochemistry applications. For ultra trace electrochemical analyses Type I (ultra pure) water is required.

**TECHNIQUES INCLUDE**

**Potentiometry**

Potentiometry measures the potential of a solution between two electrodes. This is a passive technique, affecting the solution very little in the process. The potential is then related to the concentration of one or more analytes. The cell structure used is often referred to as an electrode even though it contains two electrodes: an indicator electrode and a reference electrode (distinct from the reference electrode used in the three electrode system). Only demineralised water is used with electrodes since mineralic water contaminates will interfere with the electric potential of the electrodes. Potentiometry is usually conducted in an ion selective way with a different electrode for each ion. The most common potentiometric electrode is the glass pH electrode.

**pH measurement**

pH is a subclass of potentiometry and is used to measure the acidity or alkalinity of a liquid. Measurement of pH in pure water is problematic due to the low ionic strength of the solution and because the rapid uptake of carbon dioxide affects the observed reading.

**Coulometry**

Coulometry uses applied current or potential to completely convert an analyte from one oxidation state to another. In these experiments the total current passed is measured directly or indirectly to determine the number of electrons passed. This can indicate the concentration of the analyte or, when the concentration is known, the number of electrons involved with a redox couple. Bulk electrolysis, also known as controlled potential coulometry, or some hybrid of the two names, is perhaps the most common form of coulometry.

**Voltammetry**

Voltammetry applies a constant and/or varying potential at an electrode’s surface and measures the resulting current with a three electrode system. This method can reveal the reduction potential of an analyte and electrochemical reactivity among other things. This method in practical terms is nondestructive since only a very, small amount of the analyte is consumed at the two-dimensional surface of the working and auxiliary electrode.
Polarography
Polarography is a subclass of voltammetry that employs a dropping mercury electrode as the working electrode and often uses the resulting mercury pool as the auxiliary electrode. Concern over the toxicity of mercury, combined with the development of affordable, inert, easily cleaned, high quality electrodes made of materials such as noble metals and glass carbon, has caused a great reduction in the use of mercury electrodes.

Amperometry
Amperometry is a subclass of voltammetry in which the electrode is held at constant potentials for various lengths of time. This is mostly a historic distinction that still results in some confusion, for example, differential pulse voltammetry is also referred to as differential pulse amperometry, which can be seen as the combination of linear sweep voltammetry and chronoamperometry. One thing that distinguishes amperometry from other forms of voltammetry is that it is common to sum the currents over a given time period rather than considering them at individual potentials. This summing can result in larger data sets and reduced error. Amperometric titration is a technique that would be considered amperometry since it measures the current, but would not be considered voltammetry since the entire solution is transformed during the experiment.

SPECTROSCOPY
Spectroscopy was historically the study of the interaction between radiation and matter as a function of wavelength (λ), and it referred to the use of visible light dispersed according to its wavelength, i.e. by a prism. Later the concept was further expanded to comprise any measurement of a quantity as a function of either wavelength or frequency. Thus it also can refer to interactions with particle radiation or a response to an alternating field or varying frequency (ν). Once the very close relationship between photon energy and frequency (E=ħν) was realised, where ħ is the Plank constant, a further extension of the definition added energy (E) as a variable. A plot of the response as a function of wavelength — or more commonly frequency — is referred to as a spectrum.

Spectrometry
Spectrometry is the spectroscopic technique that is employed to assess the concentration or amount of a given substances and the instrument that performs such measurements is a spectrometer or spectrograph.

IDENTIFYING YOUR DRINKING WATER QUALITY
Over 70 years of experience in the lab water industry, combined with Veolia’s expertise in running many municipal treatment plants, gives ELGA unsurpassed knowledge about feedwater qualities throughout the world. On our first visit to your laboratory we will carry out a test, on site, to analyse your feed water quality. Armed with data about your laboratory’s water quality, required applications, lab design and budget, our sales team will deliver an informed proposal about the best water purification solutions to suit your needs.
TECHNIQUES INCLUDE

Flame Atomic Absorption Spectrophotometry (F-AAS)
Although somewhat eclipsed by ICP-MS and ICP-ES for multielement analyses, the relatively modest cost of AAS ensures its use in smaller laboratories or for specific analyses. Depending on the element, detection limits vary from low ppb to ppm levels. ASTM Type II water is usually pure enough for most routine AAS and there is no requirement for low levels of organic compounds or bacteria.

Gas Chromatography – Mass Spectrometry (GC-MS)
For GC, purified water is used to prepare blanks, standards and sample pretreatments, e.g. solid phase extraction. Since high sensitivity can be achieved in GC-MS, the requirement for water purity is extremely stringent. Very low TOC levels, i.e. less than 3 ppb, are required and this can best be achieved by using a top-of-the-range polisher that is fed with water that has been pre-treated by Reverse Osmosis for removal of ions and organic compounds.

Graphite Furnace Atomic Absorption Spectrophotometry (GFAAS) also known as Carbon Furnace Atomic Absorption Spectrophotometry (CFAAS)
This variant of AAS in which the flame is replaced with an electrically heated graphite tube or rod can achieve very high sensitivity in elemental analysis. A top of the range ASTM Type I water polisher is required that ensures ppt levels of elemental impurities, 18.2 MΩ-cm resistivity water and an extremely low TOC, typically <3 ppb. Multi-stage monitoring (see above) is the only method that guarantees this level of purity and the ultimate performance is achieved with enhanced pretreatment followed by continuous recirculation and repurification of the polished water.

Mass spectrometry
This highly sensitive technique permits trace analysis of complex mixtures and therefore requires high purity water. All sample pretreatments such as solid phase extraction and sample preparation steps require ASTM Type I (ultra pure) water, which is produced by a top of the range water ‘polisher’ system. This gives ppt levels of elemental impurities, 18.2 MΩ-cm resistivity water and an extremely low TOC, typically <3 ppb. Multi-stage monitoring (see above) is the only method that guarantees this level of purity and the ultimate performance is achieved with enhanced pretreatment followed by continuous recirculation and repurification of the polished water.

Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES)
In ICP-AES, sensitivity differs markedly for different elements, however metals, semi-metals, phosphorous and Sulphur have detection limits in the ppb (μg/l) range and requires fairly stringent water purity. A high purity Type I water system (polisher), is preferred, giving >18 MΩ-cm resistivity, however TOC requirements are generally not critical and pretreatment can be by reverse osmosis or ion exchange.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS)
Advances in modern analytical instrumentation have continued to improve the sensitivity of trace metal analysis. These elements are now measured at ppt and sub-ppt levels using techniques such as ICP-MS. Trace analytical work requires water that is free from the components to be measured and demands the same extremely stringent water purity for the most sensitive ICP-MS work.
Typically cleanroom facilities are preferred for preparing high-quality reagents for blank analysis, standard dilutions and sample preparations. The water purifying system specified should be a purpose-designed ASTM Type I system. This should include a form of multi-stage monitoring (see PureSure Diagram, page 10) to guarantee these levels of purity. Ultimate performance is achieved with enhanced pre-treatment from a recirculating ASTM Type II system.

**Spectrophotometry**

Purified water for spectrophotometric applications is recommended to be at least of Type II quality with a low level of inorganic, organic or colloidal contaminants. Typically, the water has a resistivity $>1 \text{ M}\Omega\text{-cm}$ and has been micro-filtered. Low TOC content ($<50 \text{ ppb}$) is of particular importance in techniques where UV detection systems are used, as dissolved organics may interfere with detection.

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**CHROMATOGRAPHY**

Chromatography may be preparative or analytical, but the two are not mutually exclusive. Preparative chromatography seeks to separate the components of a mixture for further use. Analytical chromatography normally operates with smaller amounts of material and seeks to measure the relative proportions of analytes in a mixture.

**High Performance Liquid Chromatography (HPLC)**

HPLC can be used for the direct analysis and determination of minor and major components in a complex mixture. In the mobile phase, purified water of general laboratory grade (ASTM Type II) with a TOC of typically $<50 \text{ ppb}$ and a resistivity $>1 \text{ M}\Omega\text{-cm}$ is used to prepare blanks, standards and for sample pre-treatment.

Gradient HPLC is capable of extremely low detection limits, e.g. well below 1 ppb, therefore blanks, standards and sample pretreatment require extremely stringent, high quality pure water (HPLC grade water), where the lowest possible TOC levels are typically less than 3 ppb (see graph). This can be best achieved with a top-of-the-range Type I water system (polisher) especially designed for the purpose, fed with Type II or Type III water pre-treated by RO (reverse osmosis).

**Ion Chromatography (IC)**

IC determines minor and major components in a range of substances down to 0.1 ppm by direct injection of 10 to 50 microlitre samples. Highly purified water is needed for blanks, standards and to prepare eluents. While ASTM Type I water is preferred, Type II+ water is often adequate, especially if price is an issue. Extremely low limits of detection (down to low ppt levels) can be achieved using IC if the ions are preconcentrated on a short ion exchange column and then eluted into the eluent stream for separation and analysis. 50 or 100 ml of sample can be analyzed in this way. A top of the range Type I (preferably Type I+) water system is essential for obtaining ppt levels of elemental impurities, 18.2 MΩ-cm resistivity ultra pure and low TOC. Multi-stage monitoring provides a guarantee of purity not offered by alternatives (see PureSure diagram, page 10). The ultimate performance is achieved with enhanced pre-treatment followed by continuous recirculation and repurification of Type I water.

**Trace gradient HPLC of primary grade and ultra pure water**

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**Time**

<table>
<thead>
<tr>
<th>Method</th>
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<tbody>
<tr>
<td>RO permeate</td>
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<td>Ultra-pure water</td>
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<td>Blank</td>
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GENER
LABORATORY
APPLICATIONS

General chemistry
Laboratory grade purified water with resistivity >1 MΩ-cm, TOC less than 50 ppb and bacterial count of <10 CFU/ml is recommended for general chemistry applications.

Glassware washing/ rinsing
Glassware washing is a routine practice in most laboratories and the grade of water required depends on the nature of the applications. To minimize costs (and depending upon your local drinking water quality), most general purpose glassware can be washed with ASTM Type III water. For more sensitive analytical or research techniques, Type II water with a resistivity of 1 to 15 MΩ-cm should be used. For critical applications, such as trace analytical techniques (e.g. ICP-MS), cell culture or stringent clinical methods (like DNA sequencing or extraction), glassware should be washed with ultra pure water, especially for the final rinse to ensure that the ultra pure buffers, media or diluents are contained in “noncontaminated” glassware. For this, Type I (ultra pure) water, inorganics should be 18.2 MΩ-cm, TOC < 10 ppb and bacterial counts <1 CFU/ml.

Qualitative analyses
Most qualitative analysis methods for major or minor constituents require general laboratory grade purified water with resistivity >1 MΩ-cm, a TOC less than 50 ppb, low particulates and bacterial counts. However, for more sensitive techniques such as ICP-MS, ultra pure water from a top of the range water polisher is required to produce ppt levels of elemental impurities, 18.2 MΩ-cm resistivity water and low TOC.

Sample dilution and reagent preparation
The water required for diluting samples, blanks, reagents and standards must be of sufficient purity that subsequent analyses are not affected. Preparing general-purpose buffers, blanks and standards for general chemistry techniques, and for analyses >1 ppm the use of a general laboratory grade purified water with a typical resistivity of >1 MΩ-cm, a TOC of <50 ppb and low bacteria will enable accurate results. For trace analysis at ppb levels or lower, ASTM Type I (ultra pure) water is required for preparing blanks and standards.

SPE – Solid Phase Extraction
This technique is widely used in trace organic determinations as a pretreatment to separate the trace components of interest from the major components of the matrix. For trace analysis water of the highest organic purity is needed to prepare blanks and standards, and to rinse the solid phase. This can best be achieved with a top of the range Type I water system that has a minimum TOC specification (especially designed for this purpose), and is fed with water pre-treated by reverse osmosis (RO). Additional operational protocols may be needed to ensure continual high performance.

Steam generators
Steam generators are used in a wide range of applications including clean room humidification, moisturisation, direct steam heating, injection and in autoclaves and sterilisers. Most steam generators benefit from pre-treatment of the water supply to avoid build-up, precipitation or contamination in order to reduce maintenance, improve performance and enhance hygiene levels. Steam generators can use ASTM Type III quality water with conductivity in the range of 1–50 μS/cm (0.02 to 1.0 MΩ-cm resistivity), which is typically produced by reverse osmosis after suitable pre-treatment. Some authorities now apply strict specifications for the water used to produce ‘pure steam’ for used in disinfection services in healthcare environments.

Total Organic Carbon (TOC) analysis
This non-specific method is capable of quantifying the overall carbon content of materials. Applications range from high levels in effluents and process streams to sub-ppb levels in ultra pure water. Samples are diluted and reagents and standards prepared with water. For high level measurement Type II water is adequate, while trace work requires Type I (ultra pure) water.

Water analysis
Water analyses are required for a wide range of different purposes, e.g. ensuring that drinking water meets current standards, checking that purification processes have been successful and environmental testing of lakes and rivers. Water analysis requires purified water for the preparation of samples, standards and blanks and it must be of sufficiently high purity that it does not interfere with the analytical techniques. These analysis applications are usually performed with ASTM Type II water with resistivity of >5 MΩ-cm, TOC <50 ppb and a bacterial count below 1 CFU/ml.

Buffer and media preparation
The grade of pure water required to prepare or dilute reagents depends on the sensitivity of the application. For many general chemistry applications sensitivity is not the primary factor, and therefore Type II water is of sufficient purity. It has the added advantage of having high purity in ionic terms and if UV and filtration technologies are incorporated with recirculation, can also ensure low levels of organic contaminants and microorganisms.
Environmental chambers and plant growth rooms
The salt concentration and bacterial quality of the water are of major concern. The removal of silica (present in some feed waters and not removed by some purification techniques) is considered important in order to avoid “dusting”, i.e. silica deposits on plants or samples. In organisations that use walk-in chambers, bacterial quality is of growing concern as contamination from airborne bacteria can threaten the results. A Type II or Type III water purification system is usually suitable, however if the level of bacteria is a concern, then the system should include full recirculation to the chamber as well as on-line UV recirculation for complete sanitization of water. The ELGA range of BIOPURE systems designed for stringent healthcare applications can be used successfully in these situations.

### Analytical and general applications

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<thead>
<tr>
<th>Technique</th>
<th>Sensitivity</th>
<th>Resistivity* MΩ-cm</th>
<th>TOC ppb</th>
<th>Filter μm</th>
<th>Bacteria CFU/ml</th>
<th>Grade of Pure Water</th>
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* At 25°C  NA - not applicable  ND - not detected  Figures in red - critical impurities
Life science applications

RESEARCH APPLICATIONS

Molecular biology
Water quality for microbiology must generally be high. Focusing on the study of nucleic acids, proteins and enzymes, molecular biology research can be seriously affected by contaminating microorganisms and associated biologically active cell debris and products. Quite apart from the use of nuclease-free water, care should be taken to ensure that an incorrect water purity does not have an effect on the salt concentrations of prepared solutions for electrophoresis and blotting, as well as the production of reagents for DNA sequencing, DNA extraction and PCR (Polymerase Chain Reaction). The effect of humic acid as a DNA inhibitor is often overlooked. All of these concerns can be dealt with in choosing a purpose built, high quality “Genetics Grade” water system which will provide water above Type I purity.

Electrophoresis
Macromolecules can be separated from one another by several different techniques, including chemical methods, ultra centrifugation and electrophoresis. The key requirement for water for electrophoresis is the absence of significant levels of biologically active molecules such as endotoxins (typically <0.005 EU/ml), nuclease and proteases (not detectable). This is best provided by ultra pure water with a resistivity of 18.2 MΩ-cm, TOC <10 ppb C, 0.1 μm or lower particle filtration, and bacterial counts below 1 CFU/ml.

Electrophysiology
Electrophysiology methods vary from measuring the biological responses to electric currents and electromagnetic fields on whole animals to studies on single cells with microelectrodes and patch clamp techniques. These techniques are often very sensitive and can yield inaccurate results if water that is relatively high in contaminating inorganics is used. Typically, ASTM Type II water with a resistivity of >1 MΩ-cm, TOC <50 ppb and <1 CFU/ml bacterial count should be employed.

Endotoxin analysis
Endotoxin specifications are set for a wide variety of water applications that include dialysis, injectables and cell culture. Maximum permitted levels range from 0.25 EU/ml (Endotoxin Units/millilitre) down to 0.03 EU/ml. For endotoxin analysis Type I (ultra pure) water is required with an appropriate endotoxin specification of typically 0.05 EU/ml or less. Filtration with ultrafilters or charged filters, preferably combined with UV photo-oxidation, will be required.

Water for histology
Cells for histology are fixed and non-viable therefore Type II water is adequately pure. Typical values are a resistivity of >1 MΩ-cm, TOC < 50 ppb and <1 CFU/ml bacterial count.

Hybridisation
– see Molecular biology

Hydroponics
The water source for hydroponics needs to be sufficiently pure to allow added concentrations of minerals and nutrients to be gauged accurately, as well as protecting against the possible indirect effects that contamination could cause. For example, high levels of dissolved elements, especially calcium and...
magnesium, can lead to high alkalinity that varies according to water depth. Sodium and chloride can also cause direct toxicity to plants at high concentrations as well as indirect damage by interfering with the uptake of calcium, magnesium, nitrate and trace elements. Type II demineralised water, with low levels of ionic, organic and bacterial contamination, is recommended for hydroponics.

**Immunocytochemistry**
The use of antibodies in immunocytochemistry for detecting the distribution of specific proteins is prone to interferences from contaminating microorganisms and associated biologically active cell debris and products, therefore apyrogenic Type I (ultra pure) water is recommended. This is produced by 'polishing' water that has been pre-purified by deionisation, reverse osmosis or distillation and then carrying out ultrafiltration to ensure the removal of nucleases and endotoxins.

**Microbiological analysis**
Routine microbiological analysis requires Type II purified water which is largely free of bacterial contamination and has low levels of ionic, organic and particulate impurities. Typical values are a resistivity of >1 MΩ-cm, TOC <50 ppb and <1 CFU/ml bacterial count.

**Monoclonal antibody research**
Monoclonal antibodies are a valuable tool in the development of new therapeutics and in-vivo diagnostic products. Media or buffers of high purity are essential for the culture of sensitive cell lines that express monoclonal antibodies. While high levels of contaminating organics, inorganics and dissolved gases can impact the culture directly or indirectly, e.g. changes in pH, the major concern for cell culture applications is the effects of contaminating microorganisms and their associated biologically active cell debris and products. Water used for culturing bacteria that are expressing monoclonal antibodies should be of at least general laboratory grade, with resistivity >10 MΩ-cm, a TOC of less than 50 ppb and a bacterial count below 1 CFU/ml. For sensitive mammalian cell culture, the use of apyrogenic, ASTM Type I water is recommended.

**Plant tissue culture**
(Micropropagation)
Micropropagation techniques allow large scale cloning of plant species and the production of disease-free plants. To minimise the effects of potentially contaminating biologically active species the use of apyrogenic Type I (ultra pure) water is recommended.

**PCR**
— see Molecular biology

**Mammalian and bacterial cell culture**
Successful cell culture requires highly pure media and buffers to ensure the cells are free from bacterial, yeast and viral contaminants. While high levels of contaminating organics, inorganics and dissolved gases can impact the culture directly or indirectly, e.g. changes in pH, the major concern for cell culture applications is the effects of contaminating microorganisms and their associated biologically active cell debris and products. Water used for bacterial cell culture is recommended to be of at least Type II quality with resistivity >10 MΩ-cm, a TOC of <50 ppb and a bacterial count below 1 CFU/ml, while more sensitive mammalian cell culture work requires apyrogenic, Type I (ultra pure) water.

**Radioimmunoassay (RIA) and Enzyme-linked immunosorbent assay (ELISA)**
The antibody reactions used in ELISA are relatively robust and typically do not require the highest purity water. Type II water with a resistivity >10 MΩ-cm, a TOC of <50 ppb and a bacterial count below 1 CFU/ml is suitable.
CLINICAL HEALTHCARE APPLICATIONS

Clinical biochemistry & immunology
(See section 2 Clinical Diagnostics)
In clinical laboratories water should comply with appropriate water quality standards; the most relevant is the Clinical Laboratory Standards Institute (CLSI) Type CLRW – see page 45. Care must be taken to ensure water is completely sterile and usable for sanitization procedures. Water used for feeding clinical analysers, or in any preparative or analytical procedure, should be of a high quality and is produced by a combination of purification technologies. While the quality of water required for clinical analysers is specified by the manufacturer, it typically would have a resistivity >10 MΩ-cm, TOC <50 ppb and bacterial levels <5 CFU/ml. However, with immunoassay featuring increasingly in automated analyser installations requirements for higher bacterial qualities are increasing. This is largely because some assays utilize enzyme markers which can be affected by by-products of bacteria present in water. A bacteria specification of <1 CFU/ml (to the analyser rather than merely the outlet of the pure water system) is needed in this case. This requires a detailed assessment of the distribution and storage of water. It is advisable to contact your local ELGA specialist for advice on this.

Endoscopy
Critical applications in healthcare can require very low (as little as 10 CFU/100ml) levels of bacteria. In some cases water with low endotoxin levels is required for rinsing of endoscopes after disinfection. ASTM Type III or Type II grade water may be used with UV, ultrafiltration and regular sanitisation. For critical applications a purpose-built system is recommended to achieve the levels of Biopurity needed (e.g. ELGA’s BIOPURE system).

Life science applications

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*At 25°C  NA = not applicable  ND = not detected  Figures in red = critical impurities
Section 2

Clinical diagnostics — specific impurities and their effects on tests

Water quality is extremely important in clinical diagnostics. Water quality that is below the accepted standards not only affects the chemistry of the tests but can also affect the general operation of the analyser which, in turn, will reduce the reliability of the test results and increase calibration times and reagent costs.
Diagrammatic view of how purified water is used in a clinical analyser

1. Cuvette wash station
   Consistent high quality water for effective cuvette washing, eliminating carry over and contamination.

2. Sample probe and wash station
   Consistent high quality water increases calibration stability and eliminates sample to sample cross contamination.

3. Pipetting Syringes
   High quality particle free water for more accurate and precise pipetting of both sample and reagent.

4. Internal reservoir
   UV and 0.2 micron filter for bacterial and particle control, reducing bacterial contamination.

5. Reagent probe and wash station
   Consistent high quality and bacterial free water delivers longer reagent stability and eliminates reagent to reagent contamination.

6. Incubator bath
   Bacteria and particle free water for accurate and precise photometric readings.

**FUNCTIONS**

Purified water can be used for many different functions in a clinical analyser, including:

- Washing reaction cuvettes
- Feeding wash stations for probes and stirrer paddles
- Diluting reagents, samples and detergents
- Incubator baths
- An interface between syringe and sample

**WATER QUALITY**

Poor water quality can affect the analyser performance in many different ways, including:

- Reduce the accuracy of pipetting volume due to particles and bacteria
- Errors in photometric readings as a result of particles interfering when a water bath is used
- Cuvette washing contamination, carryover and water marks
- Sample and reagent probe washing contamination and carryover
- Affect sample and dilution leading to errors and poor reagent stability
- As a zero standard (Ca, Mg, PO₄, HCO₃, etc.) calibration stability and sensitivity is reduced
- In immunoassay systems bacterial by-products (notably alkaline phosphatase) can interfere with some enzyme based assay results.

Perhaps the most important aspect of pure water for automated pathology analysers is reliability.

Laboratories without the budget or space for a “duplex” system, require a robust design which incorporates ‘keep you going’ systems to be used the event of an emergency or systems failure.
INTERNATIONAL STANDARDS

Since purified water is required in all industries and science-based organisations, this has led international and national standards authorities to establish water quality standards for various applications. The most relevant to the clinical analyser market is the Clinical and Laboratory Standards Institute (CLSI) formerly the National Committee for Clinical Laboratory Standards, (see Section 4: Purified Water Standards for further details).

For cases where applications are even more demanding than those already established, ELGA works with the analyser company to specify the correct water grade.


The key purified water guidelines from CLSI 3rd edition were three main types of water (Type I-III), of which Type I was most relevant to clinical laboratories and feeds to automated instruments. These have been replaced with the terms Clinical Laboratory Reagent Water (CLRW), Special Reagent Water (SRW) and Instrument Feed Water. See page 45 for more details on these grades.

Trends in clinical chemistry

Greater efficiency, productivity and improved cost effectiveness are being achieved through automating clinical chemistry procedures. Automation can now be incorporated into sophisticated sample identification, pre-analytics (sample sorting, centrifugation, de-capping, and allocation into coded secondary tubes for various on-line and off-line work stations), tracking systems for sample transfers to various users and finally a refrigerated storage system for sample retention enabling further investigation and testing.

Validation and trend monitoring

Increasingly, validation of water purification systems is becoming mandatory i.e. objective evidence has to be provided that confirms a purification system meets the requirements for a specific use or application. Purified water should be validated as “fit for its intended purposes” and the purity specifications should be incorporated into the water purification validation procedure. This is used to document the system’s ability to deliver adequate volumes of purified water at the stated specifications, as detailed in the user requirement specification.

After validating the water as “fit for purpose”, it is critical to ensure that it continues to meet the required specifications; this is achieved by measuring and documenting defined parameters at established regular intervals. Furthermore, this approach can detect deterioration of purification components before this has impacted on the required water quality. Deterioration in a measured parameter, e.g. changes in the required resistivity or TOC, indicates the need for system maintenance or further investigation, to ensure the required water specification is always met. Additionally, recording critical parameters over a defined time is essential to identify gradual changes in water quality and enable corrective measures to be taken. For example, if ion exchange cartridges are used beyond their intended life, impurities which could interfere with the test analysis reactions can be eluted into the purified water at levels that may not register on built in monitoring systems.

EFFECT OF PURE WATER REQUIREMENTS

Higher purity

Advancements in analyser technologies demand good quality water feed for maintaining high performance and reliability. Since water is used in virtually every process on an analyser, it is crucial that quality is monitored and verified to ensure the integrity of test results. Integration of multiple technologies into a single analyser to perform both chemistry and immunology...
applications results in higher quality pure water being required for more sensitive immunology testing. While smaller sample and reagent volumes reduce costs, they require higher purity water because of the increased sensitivity needed for smaller sample volumes.

**Tests**
The diagnosis or extent of certain diseases is associated with the levels of specific proteins, known as biomarkers, in the blood – for example, elevated levels of Tropinin signifies artherosclerosis, B-type natriuretic peptide (BNP) indicates coronary artery disease, AFP indicates hepatocellular carcinoma, CA-19-9 is correlated with pancreatic cancer and PSA is a marker for prostate cancer. These proteins generally occur in very low concentrations e.g. nmols/l or pmols/l and are detected by techniques that are extremely sensitive. Compared to traditional tests/assays these current detection methods have the advantage of reducing the number of tests that have to be performed.

However since they are more susceptible to contaminant interference it is crucial that the water is of the appropriate grade so that it will not contribute to this problem.

**Regulatory**
In most countries public sector laboratories are advised/regulated by an accreditation body that establishes working standards and guidelines. While this is not mandatory for private sector laboratories the significant credibility and advantages gained have resulted in more of these laboratories registering with an accreditation body, e.g. although the Collegiate of American Pathologists (CAP) is the accreditation body in the US, many laboratories in different countries also apply for CAP registration. CAP recommends that laboratory water should meet the CLSI, Clinical Laboratory Reagent Water (CLRW) grade standard as a minimum.

Clinical analyser companies are also further regulated through organisations such as the Federal Drug Association (FDA) and Medical Devices Agency. Ultimately the analyser companies are responsible for ensuring their chemistries are validated and purified water of a suitable standard is used so that all results are accurate and reproducible.

**Possible contaminants in water – sources and purification technologies**

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Section 3

Healthcare

Cleaning, sanitizing and sterilising reusable medical equipment is becoming increasingly regulated by industry guidelines and international standards as concern grows over infection control in hospitals and the spread of MRSA, hepatitis, CJD and other resistant pathogens. There are two key elements – the protection of people (patients and staff) and patient protection and the protection of equipment – to be considered in the sterilisation of reusable medical equipment:

**PATIENT PROTECTION (AVOIDANCE OF CROSS-CONTAMINATION)**

Transmissible spongiform encephalopathies (TSEs), also known as prion diseases, are a group of rare progressive conditions that affect the brain and nervous system of humans and some mammals. Causing a gradual impairment of brain function including memory changes, personality changes and problems with movement, these (presently incurable) diseases are ultimately fatal. The most common human prion diseases include, classic Creutzfeldt-Jakob Disease (CJD) and a new variant of Creutzfeldt-Jakob Disease (vCJD), both related to bovine spongiform encephalopathy (BSE).

Typically, individuals do not exhibit symptoms of the disease for many years following infection, therefore, during this incubation phase, neither they nor their healthcare providers will know that they are potentially infectious, unless they belong to a known at risk group. As the infectious agent that causes the disease is very stable and not inactivated by methods routinely used to clean and sterilise instruments, there is a small risk that transmission could take place during routine surgery on such individuals, especially where this involves contact with high risk
tissues such as brain or the central nervous system. The fight against these secondary, hospital-acquired infections, also known as nosocomial infections, and in particular, the transmission of prion-based illnesses has caused healthcare authorities and professional bodies in some countries to regulate the decontamination procedure used for medical instruments including endoscopes.

To prevent prion contamination, healthcare professionals must ensure that their instruments and endoscopes are always perfectly clean, disinfected and ready for use. The thorough cleaning of instruments is necessary to ensure that adherent infectious agents are removed together with the organic matter that protects them, to enable better contact between the disinfectant and any remaining infectious agents on the surfaces of the instrument or medical device.

PROTECTING EQUIPMENT

Inorganic contaminants such as rust, hard water deposits (scale) and residues from cleaners can, over time, damage the surface of the medical instrument and create a habitat that facilitates bacterial growth. Also heat and some disinfectants (alcohols and aldehydes) are tissue fixatives and may cause moving parts of a device to stiffen if the surfaces are not thoroughly cleaned before sterilisation/disinfection. Additional economic benefits can be demonstrated where the use of improved water quality reduces the volumes of chemical cleaners.

Typical water quality requirements include:
- Bacteria Total Viable Count of less than 10 CFU/100ml
- Endotoxin levels of less than 0.25 EU/ml
- Conductivity of less than 30μS/cm
- Rinsewater systems should be regularly disinfected and validated to ensure they continue to meet the water specification
- Water samples should be routinely taken to demonstrate compliance

These guidelines and standards were introduced to minimise the risk of cross infection to patients from a range of bacteria including Mycobacteria, Pseudomonas and Staphylococcus epidermis.

DECONTAMINATION OF ENDOSCOPES

Most surgical instruments are disinfected using a process of cleaning, thermal disinfection and sterilisation; however, endoscopes and several other instruments are thermally labile. Unable to tolerate temperatures of 60°C or above, they cannot, therefore, be thermally disinfected and sterilised.

Instead, endoscopes are sterilized using a chemical disinfection procedure and then rinsed in purified water to remove all traces of the disinfectant. After decontamination, the equipment must be handled carefully to minimize any risk of recontamination.

Recently, the International Standards Organisation has published standards (ISO 15883 part 4) relating to the requirements and tests for washer disinfectors employing chemical disinfection for thermolabile endoscopes. These standards specify the use of water which has a microbial specification of <10 CFU/100ml (tested on at least two samples) and if the medical device comes into contact with the bloodstream or other normally sterile areas of the body, then the standard requires that the final rinse water is controlled and monitored within the limits specified by national regulations (for example HTM0101 in the UK or perhaps United States Pharmacopeia ‘Water for Injection’ in some other countries). For many countries this requires an endotoxin specification of <0.25 EU/ml.

To achieve these stringent standards a water purification system that uses RO with recirculated UV and on-line endotoxin filtration is recommended. However, overall the most important aspect of the requirements is the need to use a water purification system that maintains biopurity through simple and easy sanitisation.
Section 4

Water purification overview

The Source – Production of Drinking Water

Laboratory purified water is usually produced in situ from local drinking water that has been produced by treating natural water sources. The overall requirement for producing drinking water is that it conforms to regulations and has acceptable clarity, taste and odour. Natural water is derived from upland sources, such as reservoirs, from rivers or underground aquifers; drinking water is produced by a series of steps that vary with the water source, local and national regulations, and the choice of technologies.
Variations in raw water quality
Unlike other raw materials, drinking water may vary significantly in purity both from one geographical region to another and from season to season. Water derived from an upland surface source, for instance, usually has a low content of dissolved salts and is relatively soft, but has a high concentration of organic contamination, much of it colloidal.

By contrast, water from an underground source generally has a high level of salts and hardness but a low organic content. River sources are intermediate in quality, but also often contain products from industrial, agricultural and domestic activities. Seasonal variations in water quality are most apparent in surface waters. During the autumn and winter months, dead leaves and decaying plants release large quantities of organic matter into streams, lakes and reservoirs. As a result, organic contamination in surface waters reaches a peak in winter, and falls to a minimum in summer. Ground waters are much less affected by the seasons.

The quality and characteristics of the drinking water supply determine the purification regime required to produce purified water.

Natural water quality varies with:
- Geography
- Source i.e. surface water, aquifer (underground source)
- Season

Drinking water is often
- Passed through a series of screens to remove debris, and then mixed with ozone in contact tanks to oxidise pesticides and herbicides and kill bacteria and algae
- Treated to destroy excess ozone
- Clarified to remove suspended solids, which are collected as a sludge cake (sometimes a flocculent such as poly-aluminium chloride is added to help this process)
- Sand gravity filtered and/or further ozonation
- Granular activated carbon (GAC) filtered in order to trap solid and organic matter
- Treated with chlorine to kill remaining bacteria. A small residual amount is left to maintain low bacterial levels. An extra ultrafiltration stage is also increasingly being used to remove Cryptosporidium.
IMPURITIES IN DRINKING WATER

The unique ability of water to dissolve, to some extent, virtually every chemical compound and support practically every form of life means that drinking water supplies contain many substances in solution or suspension. Many of these contaminants can affect scientific applications through their interaction with other substances – some may be the substance you are analysing.

Suspended particles

Suspended matter in water includes hard particles (sand, rock, silt, pipework debris), soft particles (vegetal debris) and colloidal particles (organic or inorganic). Suspended particles can foul reverse osmosis membranes, block fine-bore analytical columns, as well as interfere with the operation of valves and meters. Colloidal particulates give rise to haze or turbidity in the water and thereby interfere with instrument operation.

Dissolved inorganic compounds

Inorganic substances are the major impurities in water. They include:

- Calcium and magnesium salts, which cause ‘temporary’ or ‘permanent’ hardness
- Carbon dioxide, which dissolves to give weakly acidic carbonic acid
- Sodium salts
- Silicates leached from sandy river beds
- Ferrous and ferric iron compounds derived from minerals and rusty iron pipes
- Chlorides from saline intrusion
- Aluminium from dosing chemicals and minerals
- Phosphates from detergents
- Nitrates from fertilisers

Many other ions may be present depending on the natural water source. Even at trace levels inorganic ions may affect both organic and biochemical reactions by acting as a catalyst. However, demineralised water may still contain other contaminants.

Dissolved organic compounds

Organic impurities in water are mainly from biological origin. The decay of vegetal matter gives rise to byproducts that include humic and fluvic acids, tannins and lignin. Farming, paper making, domestic and industrial waste also give rise to organic compounds including detergents, fats, oils, solvents and residues from pesticides and herbicides. In addition, water-borne organics may include compounds leached from pipework, tanks and purification media. Dissolved organics can interfere with analytical techniques and affect biological experiments such as cell culture. Even slight contamination present in water used to prepare liquid chromatography eluents can cause baseline instability, decrease sensitivity and resolution and also reduce the column lifetime.

Microorganisms

Bacteria are the main microorganisms that contaminate natural water. Chlorination ensures the removal of harmful bacteria, but drinking water still contains live microorganisms, e.g. a typical bacterial level for a drinking laboratory water supply is ten colony forming units per millilitre (CFU/ml) or less. Bacteria are usually kept at low levels by employing residual levels of chlorine or other disinfectants; however, once these are removed during water purification, bacteria have the chance to proliferate. Bacteria can interfere with laboratory experiments either directly or through their by-products, such as pyrogens, alkaline phosphatase or nucleases. Sanitization of water therefore plays an important role.
Dissolved gases
Drinking water is in equilibrium with the air and so contains dissolved gases such as nitrogen, oxygen and carbon dioxide. In purified water carbon dioxide dissociates to form a weak carbonic acid (CO₂ + H₂O ⇌ H₂CO₃ ⇌ H⁺ + HCO₃⁻). This weak anion reduces the capacity of anion exchange resins. Dissolved oxygen is usually only an issue where bubble formation is a problem. Oxygen concentration can affect specific biochemical reactions, and in applications where purified water is used in open containers, it will rapidly re-equilibrate with gases in the air. Both oxygen and nitrogen can form bubbles that are detrimental to processes such as particle counting or spectrophotometer measures.

Measuring impurities in drinking water
In order to design or select a water purification system it is necessary to have information on the feedwater composition, which is usually local drinking water. The average water quality data for your building can be obtained from your local water supplier. Alternatively a sample can be taken and analysed.

Direct analysis of water

- Filter-blocking potential is estimated using a fouling index (FI) test or, less reliably, turbidity.
- Inorganic components can be determined by:
  - Ion chromatography
  - ICP-mass spectrometry
  - Spectrophotometric methods
- Electrical conductivity provides a guide to potential problems.
- Organic compounds can be determined individually, e.g. chromatographically, or an overall indication of organic content by a total organic carbon (TOC) measurement.
- Total viable bacterial counts or those of individual species can be measured by incubation in a suitable growth medium.
- Total dissolved solids (TDS) is the residue (in ppm) produced by evaporating a water sample to dryness and heating at 180°C. Since inorganic salts form the greatest proportion of the TDS residue it is used as an indicator of the total level of inorganic compounds. It can be measured directly or estimated by multiplying the conductivity of the water, in μS/cm at 25°C, by 0.7.
Methods of water purification

Water for most laboratory and clinical applications is usually purified from drinking water. The overall objective is to remove drinking water (i.e. feedwater) impurities while minimizing additional contamination from purification system components and bacterial growth. System design and component selection are critical to success. The selection of the initial stages of a purification system will depend on the characteristics of the feedwater.

The purification process starts with a pretreatment stage to reduce damage of subsequent water purification components, ensure reliable operation, and decrease the operation cost by preventing excessively frequent replacement of expensive components. The major water purification technologies are outlined below. Each has its advantages and restrictions.

**Bacteria — major water contaminants**

Microorganisms and their by-products are a particular challenge as they enter unprotected water purification systems from the feedwater, any openings in the system, or through the point of use. They will grow as biofilms on all wet surfaces of the water purification components, including storage tanks and the plumbing of a distribution system. A biofilm is a layer composed mostly of glycoproteins and heteropolysaccharides in which bacteria can multiply even when the concentration of nutrients in the water is very low, and the layer protects the organisms from periodic treatment with biocides that are primarily effective in killing planktonic (free-floating) microorganisms.

Sloughing biofilm and by-products of microorganism growth and metabolism (e.g. endotoxins) are always potential contaminants of water. The microbiological analysis of water can help to identify the problematic germs.

**The challenges for an ultra pure water purification system are to:**

- Remove the bacteria present in the feedwater
- Ensure that minimal bacteria are present in the product water
- Prevent bacteria from entering the system and re-contaminating it
- Inhibit the growth of bacteria in the system by design and periodic sanitation
OVERVIEW OF WATER PRETREATMENT TECHNOLOGIES

Microporous depth filters
Microporous depth filters are matted fibers or materials compressed to form a matrix that provides a physical barrier to the passage of particles by random adsorption or entrapment, and are characterised by nominal particle size ratings. Most raw waters contain colloids, which have a slight negative charge (measured by the Zeta potential). Filter performance can be enhanced by using micro filters that incorporate a modified surface, which will attract and retain these naturally occurring colloids, which are generally much smaller than the pore sizes in the membrane. Depth filters (typically 1-50 μm) are commonly used as an economical way to remove the bulk (> 98%) of suspended solids and to protect downstream purification technologies from fouling and clogging. They are replaced periodically.

Activated carbon (AC) – in pre-treatment media
Activated carbon is used in pretreating feedwater. It removes chlorine and chloramine to prevent them from damaging membrane filters and ion exchange resins. Most activated carbon is produced by “activating” charcoal, from coconut shells or coal, by roasting at 800-1000°C in the presence of water vapor and CO2. Acid washing removes most residual oxides and other soluble material. Activated carbon contains a maze of tiny pores with sizes that range from 500-1000 nm and a surface area of about 1000 square meters per gram. The adsorption process is controlled by the diameter of the pores in the carbon filter and by the diffusion rate of organic molecules through the pores. The rate of adsorption is a function of molecular weight and the molecular size of the organic components.

Carbon is used as either granules or molded and encapsulated cartridges, which produce fewer fine particles. Activated carbon reacts chemically with 2-4 times its weight of chlorine, and very rapidly produces chlorides; therefore even small carbon filters can effectively remove chlorine from water.

In contrast, carbon breaks down chloramines by a relatively slow catalytic reaction to produce ammonia, nitrogen and chloride; therefore, larger volumes of carbon are needed for this process. Organic fouling (the levels of which will vary from site to site) can reduce the effectiveness of carbon and this should be considered when choosing the size of carbon cylinders.

The large surface area and high porosity of activated carbons, along with material they trap, make them a breeding place for microorganisms; however, this can partially be alleviated by the addition of insoluble biocides, such as silver, to the carbon. Activated carbon beds need to be changed regularly to minimize bacterial build-up.

PURE FACTS - MICROPOROUS DEPTH FILTERS

Advantages:

- These prefilters remove >98% of suspended solids thereby protecting processes downstream from fouling and clogging
- High capacity

Restrictions:

- Not regenerable

PURE FACTS - ACTIVATED CARBON

Advantages:

- These prefilters remove chlorine and chloramine, and to some extent reduce dissolved organic contamination

Restrictions:

- Not effective in removing ions and particulates
- Need to be changed regularly to minimize bacterial build up
- Can release carbon fines
OVERVIEW OF MAJOR WATER PURIFICATION TECHNOLOGIES

Microporous depth filters are matted Reverse osmosis (RO)

RO membranes remove water contaminants that are less than 1 nm diameter and typically remove over 90% of ionic contamination, most organic contamination, and nearly all particulate contamination. RO removal of non-ionic contaminants with molecular weights <100 Dalton can be low. It increases at higher molecular weights and, in theory, molecules with molecular weights of >300 Daltons, including particles, colloids and microorganisms (also pyrogens), will be completely removed. Dissolved gases are not removed.

During the reverse osmosis of water, feedwater is pumped past the input side of a RO membrane under pressure (typically 4–15 bar, 60–220 psi) in cross-flow fashion. RO membranes are typically thin film polyamide and are stable over a wide pH range, but can be damaged by oxidizing agents such as chlorine. Pretreatment of feedwater with microporous depth filters and activated carbon is usually required to protect the membrane from large particulates, transition metals and free chlorine. Typically 15-30% of feedwater passes through the membrane as permeate and the rest exits the membrane as a concentrate that contains most of the salts, organics, and essentially all particulates. The ratio of the volume of permeate to the volume of feedwater is referred to as the “recovery”. Operating a reverse osmosis system with a low recovery will reduce membrane fouling caused by precipitation of low solubility salts. However, recoveries of up to 75% are possible, depending on the feedwater composition and filtration and softening pretreatment. The performance of the reverse osmosis component is typically monitored by measuring the percent ionic rejection, which is the difference between the conductivities of the feed and permeate divided by the feed conductivity, calculated as a %.

“ Ionic rejection” and “recovery” vary with the feedwater, inlet pressure, water temperature and condition of the reverse osmosis membrane. Reverse osmosis, with its exceptional purifying efficiency, is a very cost-effective technology for the removal of the majority of impurities. However, it is limited by the relatively slow rate of production and is, therefore, normally used to fill a reservoir prior to use or further purification. A reverse osmosis water system protects the system from colloids and organic fouling and is often followed by ion exchange or electrodeionisation.

Advantages:
• Effective removal of all types of contaminants to varying degrees (bacteria, colloids, dissolved inorganics, particles and pyrogens)
• Requires minimal maintenance
• Operation parameters – easy to monitor

Restrictions:
• Limited flow rates per surface unit require either a large membrane surface or temporary water storage
• Require good pretreatment to avoid contaminants damaging membrane:
  - Scaling: CaCO3 deposits on surface
  - Fouling: organic or colloid deposits on surface
  - Piercing: physical damage by particles
**Ion exchange (IX)**

In this process beds of ion exchange resins can efficiently remove ionized species from water by exchanging them for H⁺ and OH⁻ ions. These resins are sub 1 mm porous beads made of highly cross-linked insoluble polymers with large numbers of strongly ionic exchange sites. Ions in solution migrate into the beads, where, as a function of their relative charge densities (charge per hydrated volume), they compete for the exchange sites. Deionisation beads are either cationic or anionic and exchange either hydrogen ions for cations e.g. sodium, calcium and aluminium or hydroxyl ions for anions e.g. chloride, nitrate and sulfate. The hydrogen ion from the cation exchanger unites with the hydroxyl ion of the anion exchanger to form pure water. Strong cation resins are polysulfonic acid derivatives of polystyrene cross-linked with divinylbenzene. Strong anion resins are benzyltrimethyl quaternary ammonium hydroxide (Type 1) or benzylidimethyethyl quaternary ammonium hydroxide (Type 2) derivatives of polystyrene crosslinked with divinylbenzene.

Beds of ion exchange resins are available as cartridges or cylinders and are typically used for a period of time and then replaced, when cations and anions have replaced most of the H⁺ and OH⁻ active sites in the resins. Cylinders can be fed directly with drinking water to provide purified water on demand. When exhausted they are either returned to a regeneration station for recharging or else discarded. Greater water purity and extended resin lifetimes can be achieved by pretreating the feedwater with reverse osmosis prior to ion exchange; this approach is often used for high purity laboratory water purifiers. This also avoids fouling of the resin surface by large organic molecules, which would reduce capacity.

**PURE FACTS - ION EXCHANGE**

**Advantages:**
- Removes dissolved inorganic ions, giving a resistivity of 18.2 MΩ-cm (at 25°C); <1ppb total ionic contamination
- Regenerated by deionisation using acid and bases or electrodeionisation
- Relatively inexpensive

**Restrictions:**
- Does not effectively remove bacteria, organics, particles or pyrogens
- Finite capacity – once all ion sites are occupied, ions are no longer retained
- Chemically regenerated de-ionised beds can produce organics and particulates
- Single use resins require good quality pre-treated water to be used efficiently and economically
The very large surface areas of ion exchange resins make them a potential breeding place for microorganisms, and can lead to the release of fines and soluble components. For these reasons, good quality resins should be used, and bed volumes kept as small as reasonably possible. Filters are typically installed after the beds to trap fines and other particulate matter. Bacterial build up can be minimised by frequent recirculation of the water and by regular cartridge replacement. As ion exchange beds exhaust, they release pulses of contaminants that have accumulated from the water. Strongly bound contaminants may displace weakly bound contaminants, so the first pulses of contaminants are likely to be weakly ionised substances that will have little effect on the resistivity of the product water.

Resistivity monitoring is unlikely to detect the initial release of these weakly ionised species, including charged organics, silicates and borates. This situation is illustrated in the graph above, which shows the release of silica, and organics as TOC before the resistivity falls detectably, as an ion exchange bed begins to exhaust.

The undetected release of weakly bound ionic contaminants can be prevented by multi-stage monitoring (e.g. ELGA's PureSure), which uses two identical ion exchange resin beds in series with a resistivity monitor between them. As the first (primary) bed begins to exhaust, the released weakly ionised species are bound by the second (polishing) bed and thus are not present in the final product water. Resistivity is measured after the first stage to detect bed exhaustion. The second bed is then shifted to the first position and a new bed installed in the second position.

This strategy makes efficient use of the resin, because the first bed does not have to be exchanged until the intermediate resistivity drops below 1 MΩ·cm @25°C, which is easily determined, and the second bed will still retain virtually all of its initial capacity when it is moved to the primary position. Other, less effective, approaches include replacing beds well before they exhaust or the use of specialised resins that bind weakly ionised species tighter. With suitable choice of resin, pretreatment and system design, ion exchange enables the lowest levels of ionic contamination to be achieved.
Electrodeionisation (EDI)
Electrodeionisation is a technology that combines ion exchange resins and ion-selective membranes with direct current to remove ionised species from water. Its development and use in water purification overcame some of the limitations of ion exchange resin beds, particularly the release of ions as the beds exhaust and the associated need to change or regenerate the resins. Water passes through one or more chambers filled with ion exchange resins held between cation or anion selective membranes. Ions that become bound to the ion exchange resins migrate to a separate chamber under the influence of an externally applied electric field, which also produces the H+ and OH- necessary to maintain the resins in their regenerated state. Ions in the separate chamber are flushed to waste.

The ion exchange beds in Electrodeionisation systems are regenerated continuously, so they do not exhaust in the same way as ion exchange beds that are operated in batch mode. Also, Electrodeionisation beds typically are smaller and remain in service for longer periods. The resins used in Electrodeionisation systems can either be in separate chambers of anion or cation beads, layers of each type within a single chamber or an intimate mixture of cation and anion beads. ELGA’s laboratory EDI process utilises separate beds of cation and anion resins as well as a bed of intimately mixed resins. The separate beds of cation and anion resins are housed in wide cells that provide a flow path for the ions in transit, which offers advantages in the flexibility of design and mechanical simplicity at laboratory scale. The resin in the cells provides a buffer against changes in feedwater quality.

The quality of water produced is then further enhanced by passage through a mixed resin bed. Reverse osmosis is typically used before Electrodeionisation to ensure that the Electrodeionisation “stack” is not overloaded with high levels of salts, organics or particles. The small volume of resins in the stack results in low bleed of organic molecules. Typically, reverse osmosis removes about 95% of ions; Electrodeionisation will remove about 95% of the remaining ions as well as carbon dioxide and silica. Typically, EDI product water has a resistivity of 5 to 17 MΩ-cm (at 25°C) and a TOC content below 20 ppb. Bacterial levels are minimised because the chemical and electrical conditions within the system inhibit the growth of microorganisms. EDI will not normally provide ultra pure water with a resistivity of 18.2 MΩ-cm; however, this can be efficiently achieved by incorporating a low volume of ion exchange resin down stream of the stack. This resin has very few ions to remove and will have a very long lifetime.

**PURE FACTS - ION EXCHANGE**

**Advantages:**
- Removes dissolved inorganic ions, giving a resistivity of 5 -17 MΩ-cm (at 25°C) and a TOC content below 20 ppb
- Environmentally friendly:
  - No chemical required for regenerating resin
  - No chemical or resin disposal
  - Resins in the cells have a low “bleed” of organics and buffer against changes in feedwater quality

**Restrictions:**
- Removes only a restricted number of charged organics therefore cannot produce ultra pure water with a resistivity of 18.2 MΩ-cm
- Feedwater must be of good quality so that it does not overload the EDI stack with organics, multi-valent salts or particles. It is typically treated with RO
**Distillation**

Distillation is a long established method for water purification and separates water from contaminants by changing the state of water from a liquid phase to a gas phase and then back to a liquid phase. Each of these transitions provides an opportunity to separate water from contaminants. Water is first heated to boiling point and water vapour rises to a condenser where cooling water lowers the temperature so that the water vapour is condensed, collected and stored. In principle, distillation can remove all classes of water contaminants, with the exception of those that have vapour pressures close to water and azeotropes. Distillation is most effectively performed with pre-treated water to minimise the build up of precipitates and the carryover of impurities. Water produced by this method is called “distilled water”.

Laboratory stills are unlikely to produce adequate purification from untreated feed water, especially if precipitation occurs, so laboratory stills are most frequently fed with pre-purified water from RO or ion exchange. Laboratory stills are continuous; as boiler water is distilled away, it is replaced with fresh feed water. Careful design is essential to minimise the possible transfer of less volatile contaminants e.g. by splashing or by surface or steam entrainment. Contaminants that have vapour pressures higher than water are removed in the condenser stage of a still. Compound (multi-stage) condensers that equilibrate steam and boiling hot water in multiple, specialized compartments are necessary to remove these contaminants efficiently.

Contamination from the ambient air (e.g. dust, volatiles, etc.) must also be minimised.

Like reverse osmosis, distillation only produces purified water slowly and the distilled water must be stored for later use. Stills are very energy intensive – typically using 1kW of electricity per litre of water produced. Depending on the design of the still, distilled water can have a resistivity of around 1 MΩ-cm as CO₂ in the air dissolves in the distilled water. The distilled water will be sterile when freshly produced, however to maintain sterility, it is collected in sterile storage bottles and then autoclaved; however, once the bottle is opened it is exposed to bacteria and other airborne impurities and its purity rapidly deteriorates.

---

**PURE FACTS - DISTILLATION**

**Advantages:**
- Removes a wide range of contaminants
- Long shelf life

**Restrictions:**
- Slow at purifying water
- Certain contaminants are transmitted at varying amounts into the condensate
- Should be fed with pre-purified water
- Distilled water can be prone to re-contamination during prolonged storage, therefore requires meticulous maintenance
- Not economical or environmentally friendly – requires large amounts of electrical energy for heating and large volumes of tap water for cooling.
Activated carbon – in Purified Water
The second major application of activated carbon is in the removal of organic compounds from purified water, often in the purification loop prior to the final ion exchange bed. Activated carbon takes up water contaminants by virtue of ionic, polar and Van der Waals forces, and by surface-active attraction. Activated carbon beds are prone to release fines and soluble components into the water stream and do not remove all dissolved organic contaminants, but their use can produce a significant reduction in TOC. A purer form of activated carbon made from polymer beads is sometimes used for this application.

Microporous filters
Microporous screen filters provide a physical barrier to the passage of particles and microorganisms in purified water systems. Screen filters, characterised by absolute particle size ratings, have uniform molecular structures, which, like a sieve, retain all particles larger than the controlled pore size on their surface. Screen filters (0.05 to 0.22 μm) are typically used as close as possible to the point of use to trap microorganisms and fine particulates. Trapped particulates, including microorganisms or their metabolic products, and soluble matter, can be leached from filters and suitable maintenance (regular sanitisation and periodic replacement) is necessary to maintain desired levels of performance. Newly installed filters usually require rinsing before use to remove extractable contaminants. A microporous filter membrane is generally considered to be indispensable in a water purification system, unless it is replaced by an ultrafilter.

Advantages:
• Produce a significant reduction in TOC
• Long life attributed to high binding capacity

Restrictions:
• Does not remove all dissolved organic contaminants
• Sometimes releases fines and soluble components into the water stream

Advantages:
• Screen filters function as absolute filters that retain and remove all particles and microorganisms greater than their pore size
• Operate efficiently unless damaged
• Easy maintenance, i.e. only need to be replaced

Restrictions:
• Become blocked when the surface is covered by contaminants, therefore should be used in last purification step as a guarantee
• Does not remove dissolved inorganics, organics or pyrogens
• Cannot be regenerated
Ultrafilters (UF)
UFs are membrane filters that remove particles as small as protein macromolecules. Pores are typically from 1 to 10 nm and membranes in the form of hollow fibres are often used to give higher flow rates. They are characterised by the efficiency with which they reduce the concentration of relevant contaminants to acceptable levels. UF systems are usually installed near the outlet of a water purification system to reduce the concentration of microorganisms and large organic molecules, including nucleases and endotoxins. UF systems must be regularly sanitised and/or replaced to maintain their effectiveness. UF systems can be installed in a traditional fashion, where all the water flow is directed straight through the membrane, or in “cross flow” (tangential flow) fashion where a portion of the input water flows across the membrane surface to reduce fouling by rinsing away contaminants. UF is an excellent technology for ensuring consistent ultra pure water quality with respect to particles, bacteria and pyrogens.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Endotoxin conc (EU/ml)</th>
<th>Bacteria conc (CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Challenge</td>
<td>1000</td>
<td>2 x 10^7</td>
</tr>
<tr>
<td>Post UF-1</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Post UF-2</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Post UF-3</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Post UF-4</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Post UF-5</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Log₁₀ reduction</td>
<td>&gt;6</td>
<td>&gt;9</td>
</tr>
</tbody>
</table>

Vent filters
Hydrophobic microporous filters are often fitted to water storage containers as vent filters in order to prevent particulates, including bacteria, from entering the stored pure water. By combining absorptive media with filter media, composite vent filters (CVF) can also minimise CO₂ and organic contamination of stored water. Regular replacement is essential to maintain effectiveness.

Degassing (or De-aeration) membranes
A contactor device uses a hydrophobic membrane filter to remove gases (e.g. CO₂, O₂) from water. The water stream passes on one side of the membrane and a flush gas or vacuum removes gases from the other side of the membrane. The removal rate of a species is dependent on the permeability of the membrane, the contact area, contact time and the difference in partial pressure across the membrane.

**PURE FACTS - ULTRAFILTERS**

**Advantages:**
- Effectively removes most colloids, enzymes, microorganisms particles and endotoxins above their rated sizes, keeping them above the ultrafilter surface
- Efficient operation unless damaged
- Lifetime can be extended by a regular high speed water flush

**Restrictions:**
- Does not remove dissolved inorganic or organic substances
- Can block if presented with a high level of high molecular weight contaminants

![Germicidal lamp effectiveness curve](image_url)
Ultraviolet (UV) light

UV light is widely used as a bactericide and to break down and photo-oxidise organic contaminants to polar or ionized species for subsequent removal by ion exchange. The UV sources in laboratory water purification systems are low pressure mercury lamps that produce radiation with a wavelength of 254 nm. This has the greatest bactericidal action as it damages DNA and RNA polymerase at low doses thereby preventing replication, while higher doses are biocidal, thereby leading to sanitization and disinfection of water. UV chambers and lamps need to be designed to provide a sufficient dosage of UV to avoid production of live but inactivated microorganisms. Radiation at shorter wavelengths (185 nm) is most effective for oxidising organics as it breaks large organic molecules into smaller ionized components, which can then be removed by a downstream high purity ion exchange resin bed. Prior removal of organic ions, by initial ion exchange, optimises the effectiveness of this treatment. UV radiation at 185 nm is a highly effective oxidant and a key component in producing ultra pure water with the lowest levels of organic contaminants.

### Technologies used to control microorganisms

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Microporous filter</th>
<th>Ultrafilter</th>
<th>Reverse osmosis</th>
<th>Ion exchange</th>
<th>Activated carbon</th>
<th>Ultraviolet light</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>🌟🌟🌟🌟</td>
<td>🌟🌟🌟🌟</td>
<td>🌟🌟</td>
<td>🌟🌟</td>
<td>🌟🌟</td>
<td>🌟🌟🌟🌟🌟</td>
</tr>
<tr>
<td>Endotoxins</td>
<td>🌟🌟🌟</td>
<td>🌟🌟🌟🌟</td>
<td>🌟🌟🌟</td>
<td>🌟🌟</td>
<td>🌟🌟</td>
<td>🌟🌟🌟</td>
</tr>
</tbody>
</table>

**Key**

🌟🌟🌟 Excellent removal
🌟🌟 Good removal
🌟 Partial removal
* Initial high efficiency

---

**PURE FACTS - UV LIGHT**

**Advantages:**
- Oxidation of organic compounds (185 nm and 254 nm) to reach TOC levels < 5 ppb
- Effective bactericide treatment

**Restrictions:**
- Photo-oxidation of organics is a polishing step that can only decrease the TOC levels by a restricted amount
- No influence on ions, particles or colloids
- The water’s resistivity is decreased as a result of the CO2 released by photo-oxidation, as it produces H₂CO₃ (H⁺, HCO₃⁻)
System design

Different water purification technologies have been described in this section; each has its advantages and restrictions. Some are able to remove large fractions of several impurities, while others are excellent at removing one specific contaminant down to extremely low levels. Therefore, in order to remove all contaminants to produce the desired level of water purification for a particular application, it is necessary to use a combination of technologies.

Each system will require some pre-treatment based on the particular feedwater to remove particulates, chlorine or chloramine and, possibly, calcium and magnesium. This is preferably followed by reverse osmosis to remove virtually all colloids, particles and high molecular weight organic compounds and over 90% of ions. The resultant primary grade water, which is produced relatively slowly and stored in a reservoir, will contain some level of organic compounds, ions, bacteria and cell debris, dissolved carbon dioxide and oxygen. These stages can occur in separate units either locally or in a larger system with a loop providing water to a single laboratory or an entire building.

The water is next treated by one or more techniques depending on the required purity – ion exchange and/or EDI to remove ions, activated carbon or other absorbents to remove organic compounds, UV light to kill bacteria and/or to oxidise residual organic compounds, microfiltration to remove particles and bacteria and ultrafiltration to remove endotoxins, proteases and nucleases. Any or all of these stages can be combined in the same unit as the reverse osmosis, or separately in a “polisher”.
Effectiveness of water recirculation and repurification on bacterial contamination

Graph showing comparison between Static Tank and Recirculation Loop with Total Viable Count (CFU/ml) vs. Date.

1. Microprocessor controlled management system and water purity monitor
2. Activated carbon filter
3. UV photo-oxidation module
4. Recirculation of water through Docking Vessel reservoir to store and maintain water quality
5. Purification cartridge - ion exchange plus absorbive media
6. Dispenses 18.2 MW-cm (0.055 mS/cm) ultra pure water
7. Composite vent filter inhibits the ingress of airborne impurities
8. Reverse osmosis membrane
Storage and distribution

Storage and distribution are potential sources of contamination, particularly from bacteria. Good design and proper maintenance regimes are needed to minimise these problems. The materials chosen for construction are also critical and metals, other than stainless steel, should be avoided. There are many high purity plastics available but care needs to be taken to avoid those with fillers and additives which could leach and therefore contaminate the water.

Reservoirs should be protected from contaminants entering with suitable composite vent filters and purified water is often recirculated continuously or, intermittently, through some of the purification technologies to maintain purity.

<table>
<thead>
<tr>
<th></th>
<th>Feedwater</th>
<th>Post carbon filter</th>
<th>Post RO</th>
<th>Post UV</th>
<th>Post ion exchange</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conductivity (μS/cm)</td>
<td>50 to 900</td>
<td>50 to 900</td>
<td>1 to 30</td>
<td>1 to 30</td>
<td>0.055</td>
</tr>
<tr>
<td>Calcium (mg/l)</td>
<td>20 to 150</td>
<td>20 to 150</td>
<td>0.4 to 5</td>
<td>0.4 to 5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sodium (mg/l)</td>
<td>20 to 150</td>
<td>20 to 150</td>
<td>1 to 10</td>
<td>1 to 10</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Iron (mg/l)</td>
<td>0.01 to 0.1</td>
<td>0.01 to 0.1</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Bicarbonate (mg/l)</td>
<td>30 to 300</td>
<td>30 to 300</td>
<td>1 to 10</td>
<td>1 to 10</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Chloride (mg/l)</td>
<td>10 to 150</td>
<td>10 to 150</td>
<td>0.5 to 5</td>
<td>0.5 to 5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sulphate (mg/l)</td>
<td>1 to 100</td>
<td>1 to 100</td>
<td>0.1 to 5</td>
<td>0.1 to 5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TOC (mg/l)</td>
<td>0.2 to 5</td>
<td>0.1 to 2</td>
<td>0.05 to 0.2</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Total chlorine (mg/l)</td>
<td>0.1 to 1</td>
<td>&lt;0.1</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Bacteria (CFU/ml)</td>
<td>10 to 100</td>
<td>10 to 100</td>
<td>1 to 10</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Endotoxin (EU/ml)</td>
<td>1 to 100</td>
<td>1 to 100</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Turbidity</td>
<td>0.1 to 2</td>
<td>0.1 to 1</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
MONITORING – MAINTAINING THE PURITY OF PURIFIED WATER

It is impractical to monitor all potential impurities in purified water. Inorganic salts and dissolved organics are the major contaminants that affect most laboratory applications and, therefore, it is important that they are monitored on-line in laboratory water systems. The key rapid, on-line techniques are resistivity and TOC.

The conductivity, \( k \), represents the total contributions of the individual ions in the water and therefore provides valuable, non-specific indication of the ions in purified water. This includes all impurity ions and hydrogen and hydroxyl ions from the very slight natural dissociation of water. It is these hydrogen and hydroxyl ions that are responsible for totally pure water having a conductivity of 0.055 \( \mu \text{S/cm} \) at 25°C (a resistivity of 18.2 MΩ-cm).

For a strongly ionised salt, the value of \( k \) is approximately proportional to the concentration of the salt in solution and to the mobilities of its ions which are expressed as \( u^+ \) (cation) and \( u^- \) (anion). The values of \( u^+ \) and \( u^- \) also depend strongly on the viscosity of the solution and, therefore, on the water temperature, \( t \). For many ions, the relative temperature coefficient of \( u \) is around +2%/°C. In addition the value of the equilibrium constant for the dissociation of water, \( K_w \), is also temperature dependent and so the conductivity of pure water, can rise by up to 6%/°C.

Normal practice is to automatically correct all conductivity and resistivity values to 25°C. Resistivity and conductivity are easy and rapid to measure using an on-line conductivity cell (sensor) with cable and a meter, or display unit, with associated electronics, frequently provided with temperature compensation. The meter measures the resistance, \( R \), between the sensing electrodes of the conductivity cell.

<table>
<thead>
<tr>
<th>Typical values of conductivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>1mg/l NaCl</td>
</tr>
<tr>
<td>10mg/l NaCl</td>
</tr>
<tr>
<td>100mg/l NaCl</td>
</tr>
<tr>
<td>1mg/l HCl</td>
</tr>
<tr>
<td>10mg/l CO₂</td>
</tr>
</tbody>
</table>

Conductivity values of less than 2 \( \mu \text{S/cm} \) must be measured on-line as high-purity water rapidly absorbs contaminants from the surroundings, particularly carbon dioxide; causing its conductivity to rise. Although resistivity provides an excellent indication of the ionic quality of high purity water, it cannot indicate the presence or concentration of nonionised chemical species, nor is it sensitive to sub-ppb concentrations of ions due to equilibria with the hydrogen and hydroxyl ions from the water. When such levels are critical, individual contaminants may need to be measured using analytical techniques such as inductively coupled plasma mass spectrometry, ion chromatography and graphite furnace AAS.

### Control of impurities

<table>
<thead>
<tr>
<th>Impurity</th>
<th>Control approach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ions</td>
<td>Use of RO, ion exchange, EDI, on-line resistivity monitor</td>
</tr>
<tr>
<td>Organics</td>
<td>Use of RO, carbon, UV photo-oxidation, in-line TOC monitor</td>
</tr>
<tr>
<td>Particles</td>
<td>Use of absolute filters, occasional on-line monitoring, if needed</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Use of microfilters, UV &amp; sanitisation, off-line testing</td>
</tr>
<tr>
<td>Endotoxins</td>
<td>Use of ultrafilters, UV photo-oxidation, off-line testing</td>
</tr>
<tr>
<td>Bio-active species</td>
<td>Use of ultrafilters, UV photo-oxidation, off-line testing</td>
</tr>
<tr>
<td>Gases</td>
<td>Degassing at point of use, occasional on-line monitoring, if needed</td>
</tr>
</tbody>
</table>

Hints & Tips

Storage of purified water, that is not recirculated, should be kept to an absolute minimum in order to restrict deterioration in quality and bacterial growth.
Electrical conductivity and resistivity are both measures of a fluid’s ability to conduct electrical current. Conductivity is the reciprocal of resistivity, e.g. conductivity = 1/resistivity. The ionic content of purified water is provided by measuring electrolytic conductivity, k, and its reciprocal, resistivity, r.

\[ k = \frac{F \cdot \sum c_i z_i u_i}{r} \]

Low conductivity = high resistivity

In practice, conductivity units are typically used when referring to water ranging from raw water though to drinking water and primary grade, while resistivity units are used for ultra pure water such as deionised or reverse osmosis water.

The unit of conductivity is the Siemen (S/cm) and the unit of resistivity is Ohms (Ω-cm).

A Meg-ohm (MΩ-cm) = 1,000,000 Ohms.

Since conductivity and resistivity relate to an area between which current is measured i.e. length/area, it is typical to see the units expressed as MΩ-cm or S/cm.
pH
The measurement of pH is not recommended for pure water. High-purity water rapidly picks up contaminants that affect its pH and it also has a low conductance, which causes instability in most pH meters. Fortunately, since the concentration of hydrogen ions in the water affects both pH and resistivity, the pH must lie within certain limits for a given resistivity reading. For example, if the resistivity is 10 MΩ-cm, the pH must lie between 6.6 and 7.6.

Monitoring biologically active species
To monitor contaminating biologically active species, samples of purified water are filtered through a sterile 0.22 μm membrane filter. Bacteria present in the sample are trapped on the filter, which is then placed on the surface of a low nutrient media and incubated. The nutrients from the media diffuse through the filter allowing the growth of colonies, which are counted, typically, after 3 to 5 days.

Since this “plate counting” technique has an inherently long delay before results can be obtained it is essential to use regular bacterial counts to monitor the background long-term bacterial acceptability. This is achieved using epifluorescence microscopy of a stained, filtered sample and can be used to rapidly detect and distinguish between living and dead microorganisms, making it useful when a rapid corrective action is indicated. Epifluorescence counts are likely to differ considerably from those obtained from plate counting as microorganisms growing in laboratory water purification systems do not necessarily grow quickly, or well, on plate media. Endotoxins are lipopolysaccharides present in the cell walls of gram negative bacteria. They produce adverse effects in many molecular biological procedures and a toxic response if injected into humans. Standard tests based on Limulus Amebocyte Lysate activity are used to measure endotoxin levels. Similarly, other biologically active species such as RNase, DNase and proteases can severely interfere with many molecular biological techniques, use of nuclease free water is therefore advised. Various specific tests, often in kit form, are available for detecting these species off-line.

Procedures must be established for maintenance and/or replacement of water purification system components to ensure that the product water meets specifications at all times. Trend monitoring of parameters that measure product water specifications makes it possible to anticipate some maintenance. The frequency of maintenance activities should follow, as a minimum, the manufacturer’s recommendations.

Sanitisation of the water purification and distribution system is critical to ensure microbial contamination is controlled within specifications. Sanitisation frequency must be adequate to maintain the purity specifications and is established based on system usage, regular quality control trend data, and the system manufacturer’s recommendation. Chlorine solutions, per-acetic acid and hydrogen peroxide are often used as sanitants.

Hints & Tips
The microbiological purity of the water in a water treatment system can only be maintained by recirculating the water through the various purification processes via the storage reservoir. The storage reservoir should be sealed and fitted with a bacterial air filter or composite vent filter to prevent the ingress of airborne contamination.

Hints & Tips
Change ion exchange cartridges regularly, typically every six months, to minimise the build-up of bacterial contamination.

Hints & Tips
Use ultra-clean apparatus (glass or plastic) for work involving high-purity water. For sensitive analytical techniques, sample containers should be soaked in ultra pure water before use. Glass vessels are recommended when the organic quality is critical; these may require special preparation.

Hints & Tips
To prevent algal growth, avoid using translucent reservoirs and pipework and avoid installing storage vessels close to direct sunlight or sources of heat.
Purified Water Standards

Standards define different laboratory and water grades for both technical and economical reasons. The reason for these standards is to ensure that the right water quality is used for a specific application, while minimizing laboratory operating costs. In general, the purer the required grade of water, the more expensive it is to produce.

ELGA differentiates between four general grades of purified laboratory water:

**Primary grade**
Primary grade water has the lowest level of purity, and normally has a conductivity of 1-50 μS/cm. It can be produced by weakly basic anion exchange resins, reverse osmosis or single distillation. Weakly charged anions, such as carbon dioxide and silica may not be removed and, therefore, will be present in this water grade. Typical applications for primary grade water include rinsing glassware, feeding washing machines and humidifiers.

**Deionised**
Deionised water typically has a conductivity of 1.0 to 0.1 μS/cm (a resistivity of 1.0 to 10.0 MΩ-cm), and is produced by mixed-bed ion exchange using strongly basic anion exchange resins. It may have a relatively high and variable level of organic and bacterial contamination. It is used for a variety of purposes, including rinsing, making up general purpose analytical standards and reagents and diluting samples.

**General laboratory**
General laboratory grade water not only has high purity in ionic terms, but also low concentrations of organic compounds and microorganisms. A typical specification would be a conductivity of <1.0 μS/cm (resistivity >1.0 MΩ-cm), a total organic carbon (TOC) content of <50 ppb and a bacterial count below 1 CFU/ml. Water of this quality can be used for a multiplicity of applications, ranging from the preparation of reagents and buffer solutions to nutrient media for bacterial cell culture and microbiological studies. Laboratory grade water can be produced by double distillation or by water purification systems incorporating reverse osmosis and ion exchange or EDI and possibly with absorption and UV treatment.

**Ultra pure**
Ultra pure grade water approaches the theoretical levels of purity in terms of resistivity, organic content, particle and bacteria counts. This level of purity is obtained by ‘polishing’ water, which has been pre-purified by ion exchange, reverse osmosis or distillation. Typically, ultra pure water has a resistivity of 18.2 MΩ-cm, TOC <10 ppb C, 0.1 μm or finer particle filtration, and bacterial counts below 1 CFU/ml. Ultra pure grade water is required for a variety of sensitive analytical techniques such as trace high performance liquid chromatography (HPLC), ion chromatography (IC) and inductively coupled plasma spectrometry (ICPMS). Ultra pure apyrogenic water is required in applications such as mammalian cell culture. Ultrafiltration is used to remove any significant levels of biologically active species such as endotoxins (typically <0.005 EU/ml), nucleases and proteases (not detectable).
INTERNATIONAL STANDARDS

Since purified water is required in all industries and science-based organisations, this has led international and national standards authorities to establish water quality standards for various applications. The most relevant to the clinical analyser market is the Clinical and Laboratory Standards Institute (CLSI) formerly the NCCLS (National Committee for Clinical Laboratory Standards).

The relevant standards are:
- **Clinical and Laboratory Standards Institute (CLSI) – formerly NCCLS**
- **The International Organization for Standardization (ISO)**
- **The American society for Testing and Material (ASTM)**
- **The Pharmacopoeia including USP, EP and JP**

For cases where applications are even more demanding than those already established, ELGA will work with the company or organisation to specify the correct level and methods of purification.

The standards in this section are correct at going to press, however they are not fully quoted and since standards are regularly reviewed and updated, users should refer to the latest version of the complete standards.


The key purified water guidelines from CLSI designated three main types of water (Type I-III), of which Type I is most relevant to clinical laboratories and feeds to automated instruments.


In order to encourage users to understand the important aspects surrounding the choice of water purification systems, the CLSI has adopted a different approach in the revised guideline. It has moved from Type I, II, III designations to an emphasis on ensuring that the water is suitable for its use.

The product water meeting a set specification must be validated as fit for purpose for each laboratory procedure in which it is used. The system producing purified water must be validated to meet the user requirement specification. Regular monitoring, trending and documentation of appropriate parameters must be carried out to verify that water purification technologies and systems are working effectively.

Procedures must be established for system maintenance to keep the system in conformance with water purity specifications.

Only one grade, Clinical Laboratory Reagent Water (CLRW) is defined in detail. It can be used to replace Type or Type II water from the former guideline. The other grades, listed below are described in relation to their application and user defined details:
- **Special reagent grade water (SRW)**
- **Instrument feed water**
- **Water supplied for use as a diluent or reagent**
- **Prepackaged bottle water**
- **Autoclave and wash water applications**

<table>
<thead>
<tr>
<th></th>
<th>Type I</th>
<th>Type II</th>
<th>Type III</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria (CFU/ml) max.</strong></td>
<td>10</td>
<td>1000</td>
<td>NS</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>NS</td>
<td>NS</td>
<td>5.0 - 8.0</td>
</tr>
<tr>
<td><strong>Resistivity (MΩ-cm @ 25°C) min.</strong></td>
<td>10</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>SiO₂ mg/l max.</strong></td>
<td>0.05</td>
<td>0.1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Particulate matter</strong></td>
<td>0.2 µm filter</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Organic contaminants</strong></td>
<td>Activated carbon, distillation or reverse osmosis</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>
Clinical Laboratory Reagent Water (CLRW)
CLRW water is expected to satisfy the requirements of most routine clinical laboratory testing. The limits specified for the parameters must be met at the point where the water exits a purification system for storage or use. The specifications are intended to monitor critical parameters to adequately ensure purified water for the specific clinical laboratory testing procedures. It is mandatory that the final product water meets the impurity specifications, and that the parameters are monitored on a regular basis for trends that would indicate deterioration in the water purification process.

An important aspect regarding standards is highlighted in the CLSI guidelines. It emphasises that prescribed standards can only be indicators of what is likely to be an acceptable grade of pure water. It is the responsibility of the analyser manufacturer to ensure that a particular grade or specification of purified water is suitable for the specific chemistry application on a particular analyser.

Since chemistry, etc. can be modified, or new parameters introduced, the only ‘safe’ option is to provide the best quality water for all applications. Even then for certain chemistries specific impurities must be highlighted if they have been shown to affect the results.

Special Reagent Water (SRW)
Required for special clinical laboratory testing, special reagent water is pure water with different and usually higher purity specifications from CLRW. The specification should include the same parameters as CLRW with additional ones if required. It may be necessary for a laboratory to have a number of different SRWs. In most cases a SRW is qualified as fit for purpose for an application by testing during assay development using techniques such as specimen blank response, reagent blank response, standards additions, and interference testing. Once qualified, the laboratory needs to define the specifications and validation testing to ensure the water meets its specialised clinical testing requirement. Common applications for a SRW include:

- Trace organic analysis, which may require a lower TOC or a UV spectrophotometric absorbance specification
- DNA and RNA testing which typically requires DNase, RNase and protease free water.
- Trace metals analysis which requires a negative blank response for each metal to be measured
- Low endotoxin water (0.25 EU ml or lower) may be necessary for sensitive molecular biological applications such as cell culture, organ testing, and fluorescent antibody detection of microorganisms
- Low CO2 water may be required to prepare standard buffers for pH calibration

Instrument feed water
Instrument feed water is intended for the internal rinsing, dilution and water bath functions of automated instruments. Use of CLRW for this application must be confirmed with the manufacturer of a specific instrument and water of this specification must be used.

CLSI emphasises ensuring that laboratory water is suitable for its use. The purified water must be validated as fit for purpose for each laboratory procedure in which it is to be used. The system producing purified water must also be validated. Regular monitoring, trending and documentation of appropriate parameters must be carried out to verify that water purification technologies and systems are working effectively. Procedures must be established for system maintenance. Only one grade, Clinical Laboratory Reagent Water (CLRW) is defined in detail. Other grades are described in relation to their application and user-defined in detail.

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**Specifications for CLRW**

- **Ionic Impurities** – Resistivity 10 MΩ-cm
- **Organic Impurities** – TOC < 500 ppb
- **Microbiological Impurities** – Total heterotrophic plate count < 10 CFU/ml*
- **Particle Content** – In-line 0.2 µm filter or finer near the output stage

*epifluorescence and endotoxin testing are optional to provide extra information
International Organization for Standardization specification for water for laboratory use ISO 3696: 1987
This standard covers three grades of water as follows:

**Grade 1**
Essentially free from dissolved or colloidal ionic and organic contaminants. It is suitable for the most stringent analytical requirements including those of high performance liquid chromatography (HPLC). It should be produced by further treatment of grade 2 water, for example by reverse osmosis or ion exchange, followed by filtration through a membrane filter of pore size 0.2 μm to remove particle matter, or re-distillation from a fused silica apparatus.

**Grade 2**
Very low inorganic, organic or colloidal contaminants and suitable for sensitive analytical purposes including atomic absorption spectrometry (AAS) and the determination of constituents in trace quantities. Can be produced by multiple distillation, ion exchange or reverse osmosis followed by distillation.

**Grade 3**
Suitable for most laboratory wet chemistry work and preparation of reagent solutions. Can be produced by single distillation, ion exchange or reverse osmosis. Unless otherwise specified, it should be used for ordinary analytical work.

### Parameter Table

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH value at 25°C inclusive range</td>
<td>N/A</td>
<td>N/A</td>
<td>5.0 to 7.5</td>
</tr>
<tr>
<td>Electrical conductivity µS/cm 25°C, max.</td>
<td>0.1</td>
<td>1</td>
<td>5.0</td>
</tr>
<tr>
<td>Oxidizable matter Oxygen (O₃) content mg/l max.</td>
<td>N/A</td>
<td>0.08</td>
<td>0.4</td>
</tr>
<tr>
<td>Absorbance at 254 nm and 1cm optical path length, absorbance units, max.</td>
<td>0.001</td>
<td>0.01</td>
<td>Not specified</td>
</tr>
<tr>
<td>Residue after evaporation on heating at 110°C mg/kg, max.</td>
<td>N/A</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Silica (SiO₂) content mg/l, max.</td>
<td>0.01</td>
<td>0.02</td>
<td>Not specified</td>
</tr>
</tbody>
</table>

**American Society for Testing and Materials (ASTM) D1193-06 Standard specification for Reagent Grade Water**
This specification covers requirements for water suitable for use in methods of chemical analysis and physical testing, the choice of one of the various grades being designated by the method or the investigator.

When bacterial levels need to be controlled, reagent grade types should be:

### Bacterial Level Table

<table>
<thead>
<tr>
<th></th>
<th>Type A</th>
<th>Type B</th>
<th>Type C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total bacterial count max. CFU/ 100 ml</td>
<td>1</td>
<td>10</td>
<td>1000</td>
</tr>
<tr>
<td>Endotoxin max. EU/ml</td>
<td>0.03</td>
<td>0.25</td>
<td>*</td>
</tr>
</tbody>
</table>
Pharmacopoeia standards
Separate pharmacopoeias are produced by a number of authorities, notably in the USA, Europe and Japan. Each specifies materials, including water, to be used in medical work. The general purity level of water specified is similar in each case but differs in detail. Extra criteria are set for water required for sterile applications. The standards for purified water given in the European Pharmacopoeia (EP) and in the US Pharmacopoeia (USP) are summarised below. Water for injection into humans or other animals has stringent bacterial/pyrogen criteria and methods of preparation are specified.

Pharmacopoeia requirements for ‘purified water’

<table>
<thead>
<tr>
<th>Properties</th>
<th>EP</th>
<th>USP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conductivity</td>
<td>&lt;4.3 μS/cm at 20°C</td>
<td>&lt;13 μS/cm at 25°C</td>
</tr>
<tr>
<td>TOC</td>
<td>&lt;500 μg/l C</td>
<td>&lt;500 μg/l C</td>
</tr>
<tr>
<td>Bacteria (guideline)</td>
<td>&lt;100 CFU/ml</td>
<td>&lt;100 CFU/ml</td>
</tr>
<tr>
<td>Nitrates</td>
<td>&lt;0.2 ppm</td>
<td>-</td>
</tr>
<tr>
<td>Heavy metals</td>
<td>&lt;0.1 ppm</td>
<td>-</td>
</tr>
</tbody>
</table>

Suggested maximum values for contaminants in feed to large steam sterilisers (EN285)

<table>
<thead>
<tr>
<th>Feed water</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Residue on evaporation</td>
<td>≤ 10 mg/l</td>
</tr>
<tr>
<td>Silicate (SiO₂)</td>
<td>≤ 1 mg/l</td>
</tr>
<tr>
<td>Iron</td>
<td>≤ 0.2 mg/l</td>
</tr>
<tr>
<td>Cadmium</td>
<td>≤ 0.005 mg/l</td>
</tr>
<tr>
<td>Lead</td>
<td>≤ 0.05 mg/l</td>
</tr>
<tr>
<td>Rest of heavy metals except iron, cadmium, lead</td>
<td>≤ 0.1 mg/l</td>
</tr>
<tr>
<td>Chloride (Cl)</td>
<td>≤ 2 mg/l</td>
</tr>
<tr>
<td>Phosphate (P₂O₅)</td>
<td>≤ 0.5 mg/l</td>
</tr>
<tr>
<td>Conductivity (at 25°C)</td>
<td>≤ 5 μS/cm</td>
</tr>
<tr>
<td>pH value (degree of acidity)</td>
<td>5 to 7.5</td>
</tr>
<tr>
<td>Appearance</td>
<td>Colourless clean without sediment</td>
</tr>
<tr>
<td>Hardness (Ions of alkaline earth)</td>
<td>≤ 0.02 mmol/l</td>
</tr>
</tbody>
</table>

NOTE Compliance should be tested in accordance with acknowledged analytical methods.

European standard EN285
European standard EN285:2006+A1:2008 specifies requirements and the relevant tests for large steam sterilisers having a chamber volume of at least 60 L. These are primarily used in healthcare for the sterilisation of medical devices and their accessories and also during the commercial production of medical devices. It suggests maximum values for contaminants in the water feeding such units.
Glossary
Absorption  A process by which a substance is taken up chemically or physically in bulk by a material (absorbent) and held in pores or interstices in the interior.

Activated Carbon  A highly porous form of carbon used for sorption of organics and removal of chlorine and chloramine.

Adsorption  Adherence of molecules, atoms and ionised species of gas or liquid to the surface of another substance (solid or liquid) as the result of a variety of weak attractions.

Anion Exchange Resin  An ion exchange resin with immobilized positively charged exchange sites, which can bind negatively charged ionised species, anions.

Azeotrope  A blend of two or more components with equilibrium vapor phase and liquid phase compositions that are the same at a given temperature and pressure.

Bactericide  A chemical or physical agent that kills bacteria.

Biocide  A chemical or physical agent that kills microorganisms.

Biofilm  A layer of microorganisms enclosed in a glycoprotein polysaccharide matrix, which are adherent to each other and/or to surfaces.

Carbon Fines  Very small particles of carbon that may wash out of an activated carbon bed.

Cartridge  A pre-packed disposable container for housing a water purification resin, media or membrane.

Cation Exchange Resin  An ion exchange resin with immobilized negatively charged exchange sites, which can bind positively charged ionised species (cations).

Colloid  A stable dispersion of fine particles in water that have a typical size less than 0.1 μm. Colloids containing iron, aluminium, silica and organics are commonly found in natural and potable waters.

Concentrate  The liquid containing dissolved and suspended matter that concentrates on the inlet side of a membrane and flows to drain.

Condenser  The stage of a distillation system that removes sufficient heat from a vapourised liquid to cause the vapour to change to a liquid phase.

Conductivity  Conductivity is the reciprocal of resistivity. For water purification systems, conductivity is usually reported as microSiemens per centimeter (μS/cm) at 25°C.

Degassing  The removal of O₂ and CO₂ from water, usually by transfer across a hydrophobic membrane. CO₂ is removed to increase down stream ion exchange capacity.

Deionisation (DI)  Removal of impurity ions from water. Usually used to refer to ion exchange – see Ion Exchange.

Distillation  A purification process that takes advantage of changing the phase of a substance from liquid to vapour and back to liquid usually at the boiling temperature of the substance, in order to separate it from other substances with higher or lower boiling points.

Electrodeionisation (EDI)  Technology combining ion exchange resins and ion-selective membranes with direct current to remove impurity ionized species from water.

Endotoxin  A thermally stable lipopolysaccharide component from the cell wall of viable or non-viable gram-negative microorganisms. Can act as a pyrogen.

Endotoxin Units (IU/ml or EU/l)  A quantification of endotoxin levels relative to a specific quantity of reference endotoxin. 1 EU/ml is approximately equal to 0.1 ng/ml.

Epifluorescence  Method of fluorescence microscopy which can be used to detect bacteria after filtration and staining.

Feedwater  The water that is introduced into a purification process.

Filtration  A purification process in which the passage of fluid through a porous material results in the removal of impurities.

Fines  particulates released from a bed of material such as ion exchange resins.

Fouling Index  See Silt Density Index.

Gram-negative  refers to bacteria that do not absorb a violet stain originally described by Gram.

Hardness  The scale-forming and lather-inhibiting qualities of some water supplies, caused by high concentrations of calcium and magnesium. Temporary hardness, caused by the presence of magnesium or calcium bicarbonate, is so called because it may be removed by boiling the water to convert the bicarbonates to the insoluble carbonates. Calcium and magnesium sulfates and chlorides cause permanent hardness.

Ion  Any non-aggregated particle of less than colloidal size possessing either a positive or a negative electric charge.

Ion Exchange (IX)  The process of purifying water by removing ionized salts from solution, by replacing hydrogen ions for cation impurities and hydroxyl ions for anion impurities.

Line Cell  An electrode assembly inserted into a water stream by which the conductivity or resistivity is measured.
**Microorganism** Any organism that is too small to be viewed by the unaided eye, such as bacteria, viruses, molds, yeast, protozoa, and some fungi and algae.

**Off-line** In water monitoring systems, referring to measurement devices that are not directly coupled to the water stream.

**On-line** In water monitoring systems, referring to measurement devices directly coupled to the water stream.

**Particulates** Discrete quantities of solid matter dispersed in water.

**Permeate** The purified solution which has been produced by passage through a semi-permeable reverse osmosis membrane.

**pH** A measure of the acidity or alkalinity of a solution equal to \(-\log [H^+]\).

**Photo-oxidation** See Ultra Violet (Photochemical) Oxidation.

**Planktonic** Used to describe aquatic microorganisms that float.

**Point of Use** A dispense point from a purified water system from which water can be taken.

**Polishing** The final treatment stage(s) of a water purification system.

**PPB** Parts per billion is a unit equal to microgram per kilogram of water. Numerically ppb are equivalent to microgram per liter in dilute aqueous solutions.

**PPM** Parts per million is a unit equal to milligram per kilogram of water. Numerically ppm are equivalent to milligram per liter in dilute aqueous solutions.

**PPT** Parts per trillion is a unit equal to nanogram per kilogram of water. Numerically ppt are equivalent to nanogram per liter in dilute aqueous solutions.

**Pyrogen** A category of substances, including bacterial endotoxins, which may cause a fever when injected or infused.

**Regeneration** The method by which exhausted ion exchange resins are reactivated by treatment with strong acid or alkali.

**Resistivity** The electrical resistance between opposite faces of a one-centimeter cube of a given material at a specified temperature. Resistivity is the reciprocal of conductivity. For water analysis, resistivity is usually reported in megohm-centimeters (MΩ-cm) and corrected to the value at 25°C. All resistivity values referred to in this guide are at 25°C unless otherwise stated.

**Reservoir** In water purification systems, a container holding quantities of purified water.

**Reverse Osmosis (RO)** A process in which water is forced under pressure through a semipermeable membrane leaving behind dissolved organic, dissolved ionic and suspended impurities.

**Sanitisation** Chemical and/or physical processes used to kill microorganisms and reduce contamination from microorganisms.

**Silt Density Index** also called the Fouling Index (FI) is a test used to estimate the potential of the water to block filters, derived from the rate of blockage of a 0.45 μm filter under standard conditions.

**Softening** A water treatment process whereby cations, notably hardness-forming calcium and magnesium ions, are exchanged for sodium using cation exchange resins in the sodium form.

**Sterilisation** Destruction or removal of all living microorganisms.

**Total Dissolved Solids (TDS)** A measure of the total of organic and inorganic salts dissolved in water, obtained by drying residue at 180°C.

**Total Organic Carbon (TOC)** Total concentration of carbon present in organic compounds.

**Turbidity** The degree of cloudiness of water caused by the presence of suspended particles or colloidal material. Turbidity reduces the transmission of light and is measured in Nephelometric Turbidity Units (NTU).

**Ultrafiltration** A process in which water is filtered through a polymeric membrane having a very fine pore structure.

**Ultra-violet (Photochemical) Oxidation** A process using short wavelength light to cleave or oxidise organic molecules.

**Validation** Confirmation, through the provision of objective evidence, that requirements for a specific intended use or application have been fulfilled.
Further reading


Many of the ASTM standards are relevant to purified water (www.astm.org).

Information on water treatment can be found at www.groupve.com and www.elgalabwater.com
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