

Pure Water for the Modern Clinical Laboratory

Summary

Clinical grade water is clearly essential in a clinical laboratory and the trend is certainly towards higher purity requirements to meet the range of analyses carried out. Poor water quality not only affects the tests themselves directly but also impacts on all aspects of analyzer operation. Good water purification

design, especially providing recirculation through the key purification technologies, is the key to effective and long-term bacterial control, which is the most challenging aspect of water purification. This approach, combined with duplex operation and good support will provide the water needed.

Introduction

Water purity has always been important in clinical diagnostics but on-going developments in the approach to clinical testing and in the sophistication and range of tests available have made water purity even more critical.

The key requirements for water for clinical analyzers are:

- Highly reliable, uninterrupted supply
- Consistent high purity
- Low running costs
- Easy operation and minimal user intervention

Traditionally, analyzer feed water systems were mainly for chemistry-based analyzers that used principally colorimetric and ion selective electrode technologies. The overall trend towards less invasive technologies has led to smaller blood and other samples and their use for a wider range of tests. 500µl samples have to provide material for the initial tests and with some in reserve for retests or further studies. This has become feasible with improvements in pipetting techniques which enable high accuracy at volumes of only a few micro-liters. Sample dilution with clinical grade water prior to assay puts extremely high demands on the water purity as the effects of any contaminants are immediately multiplied by the dilution factor. Limited samples may also restrict scope for retesting,

adding extra clinical pressure to the financial pressures of ensuring that reliable results are obtained first time.

Increasingly, automated immunoassay systems have been introduced into routine clinical chemistry labs. These tend to use disposable cuvettes and sample pipette tips but still need pure water for reagent probe tip washing, pipetting and dilution of bulk reagents. Generally a higher quality of water is required for immunoassay because of the highly sensitive technologies used, such as chemiluminescence, either direct or indirect, or time-resolved enzymatic fluorescence. Many tests rely on multi-stage processes using enzymes such as alkaline phosphatase. Any drop in water purity can seriously impact on the quality and reliability of these assays.

Currently automation within clinical chemistry labs has progressed to group all the high volume tests together onto common platforms irrespective of the measuring technology. It is fairly commonplace now to use systems like the Abbott Diagnostics ARCHITECT® ci8200 and the Roche cobas® 6000 platform, both of which combine immunoassay and chemistry-based tests. The water feeding these systems tends to be used by both types of technology, and so all the water has to be pure enough for the more sensitive immunoassay technologies.

Uses of Clinical Grade Water

1 Cuvette wash station

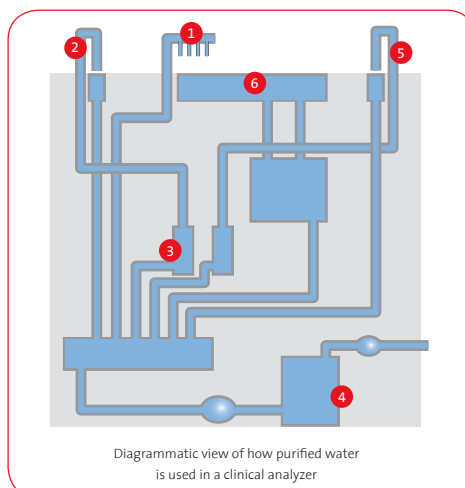
Consistent high quality water for effective cuvette washing, eliminating carry over and contamination

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

Figure 1: Uses of Pure Water

Uses of Clinical Grade Water

Poor water quality not only affects the tests themselves but also the general operation of the analyzer, which will reduce the reliability of the test results. Water is used in virtually all the processes within the analyzer:

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

Standards for Clinical Grade Water used in Clinical Laboratories

Many laboratories are moving to accreditation, which is often available locally. However, the College of American Pathologists (CAP) accreditation is used in the USA and in laboratories in many other countries. The CAP recommendation is that, as a minimum, water in the laboratory should meet Clinical Laboratory Reagent Water (CLRW) as specified by CLSI (1).

Clinical Laboratory Reagent Water (CLRW) specification is:

| | |
|----------------------------|----------------------------|
| Bacteria | <10 CFU/ml |
| Resistivity | >10 MΩ-cm |
| Total Organic Carbon (TOC) | <500 ppb |
| Particles | 0.2µm filtration or better |
| Silica (SiO ₂) | 50 ppb (Type I for CAP) |

CLSI (1) also states that for instrument feed water – “Use of CLRW for this application must be confirmed with the manufacturer of a specific instrument. Water meeting the manufacturer’s specifications must be used.” CLSI clearly anticipate that CLRW may not be pure enough for all analyzer feeds. The emphasis is on the analyzer company to validate their chemistries and use clinical grade water of a suitable purity to give accurate and reproducible results.

Effects of Impurities in the water

The CLRW specification limits four key types of impurity in pure water – ions, particulates, organics and bacteria and bacterial by-products. All will impact on analyzer performance, either by direct interference with the chemistries of tests or indirectly, by introducing errors in the measurements. General types of interference are shown in Table 1 and some examples of potential chemical interferences on specific tests are given in Table 2.

| Water impurity cause | Effect |
|---|---|
| Particles or bacteria contamination | Reduced accuracy of pipetting volume |
| Particles or bacteria contamination in water bath | Photometric reading errors |
| Wash-water contamination with ions, organics or bacteria | Cuvette contamination/carry-over |
| Wash-water contamination with ions, organics or bacteria | Sample and reagent probe contamination |
| Diluent contamination with ions, organics or bacteria | Errors in sample and reagent dilution |
| Diluent contamination with ions, organics or bacteria | Poor reagent stability |
| Zero standard water contamination (e.g. with Ca, Mg, PO ₄ , HCO ₃) | Reduced calibration stability and sensitivity |
| Ionic contamination producing low resistivity | Incorrect level sensor operation leading to reagent wastage |
| Particles or bacteria contamination or insoluble compounds | Capillary blocking |
| Insoluble deposits – contamination with low soluble compounds | Scaling |

Table 1: Effect of Impurities in Water

APPLICATION NOTE

| Clinical Test | Interferent present in water | Water purity parameter affected |
|----------------------------|---|--|
| Total Calcium | Oxalate, Sulphate, Calcium | Resistivity, TOC Resistivity |
| Alkaline Phosphatase | Fluoride, Phosphate, Zinc Manganese, Arsenate EDTA Bacteria Endotoxin | Resistivity Resistivity Resistivity, TOC TVC Endotoxin |
| Creatine Kinase (CK) | Oxidizing agents | Resistivity, TOC |
| Amylase | Oxalate, Citrate and EDTA | Resistivity, TOC |
| Lactate Dehydrogenase | Urea | TOC |
| Phosphorous | Citrate and Oxalate | Resistivity, TOC |
| Urea Nitrogen | Citrate | Resistivity, TOC |
| Iron | EDTA, Oxalate Fluoride | Resistivity, TOC Resistivity |
| Triglycerides | Glycerol | TOC |
| LDH | Hydrogen Peroxide | (Resistivity) Specific test |
| Peroxidase-based reactions | Hydrogen Peroxide | (Resistivity) Specific test |

Table 2: Examples of Chemical Interferences

Water Purity Needed

The CLRW resistivity specification of $>10\text{M}\Omega\text{-cm}$ restricts the concentrations of ionic impurities to ppb levels or less and, in effect, requires the elimination of carbon dioxide. This is adequate for most clinical work including general chemical, electrolyte, lipid and protein assays, enzymology, enzyme immunoassay, toxicology and therapeutic drug monitoring and, more recent, molecular biological techniques. When trace elements need to be determined, the water resistivity needs to be much higher – at $18.2\text{M}\Omega\text{-cm}$.

The absence of particulates is a general requirement for all types of application and is especially critical with the low liquid volumes used in modern assays. Particles can clog needles and sample handling manifolds. Deposits of particles encourage the formation of bio-film and bacterial growth and can affect the transmissivity and path length of spectroscopic cells. CLRW relies on filtration to remove particulates. However, the $0.2\mu\text{m}$ filters specified may not always be adequate.

Similarly, the TOC spec of $<500\text{ppb}$ in CLRW is a reflection of earlier standards and allows scope for the presence of a wide variety of organic compounds such as carboxylic acids and polyaromatics, which could jeopardize assays. Carboxylic acids can interfere with enzymology and enzyme immunoassays by binding to active sites, and complexing with co-factor metals. Other organics can inhibit enzymes and affect fluorescent detection.

Bacterial contamination has serious effects on all aspects of analyser operation. The key is achieving consistently low levels. For example, problems can arise in immunoassay due to fluorescein released from bacteria (e.g. *Pseudomonas aureuginosa*) giving high blanks and out-of-range standards during calibration and false positives with samples.

CLSI emphasizes the need for rigorous trending of water system parameters to ensure that water purity is achieved and maintained. Water must be validated as fit for purpose and water purification system validation is strongly recommended.

| Technology | Ions | Silica/Particles | Organics | Bacteria | Bacterial byproducts |
|-------------------|------|------------------|----------|----------|----------------------|
| General Chemistry | ✓ | | | ✓ | |
| Enzymes | ✓ | | ✓ | ✓ | ✓ |
| EIA | ✓ | | ✓ | ✓ | ✓ |
| Trace elements | ✓ | | | ✓ | |
| Toxicology (TDM) | ✓ | | ✓ | ✓ | |
| Molecular testing | ✓ | | ✓ | ✓ | ✓ |
| Analyzer | | ✓ | ✓ | ✓ | |

Table 3: Water Purity Requirements for Analyzer Technologies

APPLICATION NOTE

The water purity needs of the main analyzer technologies are summarized in Table 3. However, as discussed earlier, smaller sample and reagent volumes and the greater integration of technologies to perform both chemical and immunological tests have resulted in a general requirement for highly clinical grade water, capable of meeting all standard analyzer specifications, as shown in Table 4.

| Impurity | Parameter | Water purity needed |
|-----------|-------------|---------------------|
| Ions | Resistivity | >10 MΩ-cm |
| Organics | TOC | <30 ppb C |
| Bacteria | TVC | <1 CFU/ml |
| Particles | Filtration | 0.2 μm or better |

Table 4: Recommended water purity for clinical applications.

Water Purification Technologies

To achieve this purity virtually all the impurity ions and organic molecules must be removed from the feed water and the growth of bacteria minimized. This is best achieved with a series of purification technologies as illustrated in Figure 2.

Typical clinical laboratory water purification system

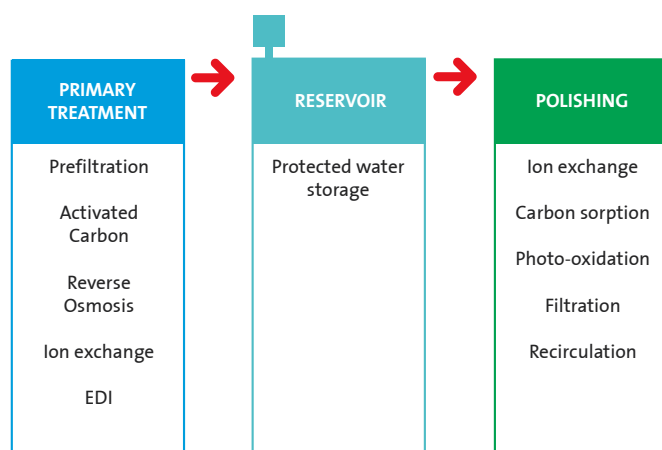


Figure 2: Multi-stage Water Purification System for the Clinical Laboratory

In the first stage of pre-treatment particles in the feed-water are removed by filtration and disinfection residues, such as chlorine and chloramines, are reacted with activated carbon.

Reverse osmosis (RO) is now the established method of removing the great majority of impurities. It is a membrane technique in which over 95% of ions are rejected along with virtually all particulates, colloids, bacteria and organic molecules with higher mass. However, it produces clinical grade water relatively slowly and its product water (permeate) is usually stored in a reservoir.

To meet CLRW or better the water needs to be purified further, usually by exposure to UV light and micro-filtration to maintain very low levels of bacterial contamination and passage through beds of ion-exchange resins which reduce ionic contamination to extremely low levels. Typical purity levels of the water after each of these stages is illustrated in figure 3.

For heavy usage applications, to markedly reduce the frequency at which the polishing ion exchange packs need to be changed and achieve a more consistent water purity, it is possible to include an electrodeionization (EDI) stage to remove most of the ions from the RO permeate before it is stored in the reservoir. EDI uses beds of ion-exchange resin which are continuously regenerated by low-voltage electrical current.

Having achieved high purity water the challenge is to keep it pure and to do so with the minimum operator intervention. The key is system design with attention to detail – the use of a well proven design with tried and tested components such as finer 0.05µm filters to give the extra level of protection against smaller particulates.

Experience with all types and scale of water purification systems has demonstrated the need for periodic recirculation of clinical grade water through the purification technologies. Using any type of reservoir will lead to bacterial growth. Recirculation through an in-line filter and UV light will remove bacteria enabling a very low steady-state level to be achieved. This will also minimize further bacterial growth by reducing the organic matter available. Relying solely on point-of-use filters can allow a microbiological “soup” and extensive biofilm to grow within the system with only a single barrier to prevent serious product water contamination. Rapid monitoring of the effectiveness of such a barrier prior to use is not possible. Recirculation is recommended by CLSI – “recirculation should include enough water purification processes to maintain a consistent level of purity in the loop” - as is periodic sanitization - “biofilm will develop...and sanitization is the only way to combat it”, “Sanitizing storage and distribution systems must be performed often enough to prevent a significant build-up of biofilm”. It is essential to make sanitization as easy and as least-disruptive as possible.

As well as direct interference effects, bacteria are a source of a range of by-products, such as alkaline phosphatase, endotoxin, RNase and DNase, which can affect assays in their own right. Their presence is a result of poor bacterial control and they

are best minimized by maintaining very low bacterial levels by active recirculation. For example, a bacterial level of <1CFU/ml will give about 0.5 micro-units of alkaline phosphatase per litre – far too low to interfere with assays.

Because of the dependence of high volume analyzers on a continued supply of high quality pure water, the purification system should incorporate an emergency bypass to allow controlled shut-down in the event of a problem and, preferably, contingency measures need to be in place, such as duplex water units and duplex analyzer feed loops to each analyzer.

As well as needing pure water to feed analyzers there are a variety of other requirements for clinical grade water in the clinical laboratory. The general applications can be more than adequately met by CLRW and can be provided by the analyzer feed systems. Special requirements, such as for trace elemental analysis or nucleic-acid-based assays, may be met with specific water purification systems. Molecular diagnostics requires type I nuclease-free water suitable for gene sequencing. To avoid interferences, this must be kept free from calcium, magnesium, organics, endotoxin and bacterial nucleases using further purification technologies, such as multiple ion-exchange, dual wavelength photo-oxidation and ultrafiltration. Chromatographic techniques, for example, LCMS-MS, GC-MS and HPLC in toxicology, require type I water with the lowest possible levels of organic contamination, best achieved by optimal system design with high purity components and dual wavelength photo-oxidation. ICP-MS and ultra-trace IC require water that is virtually free of elemental and ionic impurities, needing a high purity water system with multi-stage removal of ions using the highest efficiency and purity ion-exchange resins.

The importance of the partnership of the customer, diagnostic company and pure water unit provider is even more important than ever. The customer must be confident not only in the system itself but in the ability of the water company to maintain, trouble shoot, supply consumables and spare parts throughout the world, at any time.

Conclusion

Clinical grade water is clearly essential in a clinical laboratory and the trend is certainly towards higher purity requirements to meet the range of analyses carried out. Less pure water impacts on all aspects of analyzer operation. Good water purification design, especially providing recirculation through the key purification technologies, is the key to effective and long-term bacterial control, which is the most challenging aspect of water purification. This approach, combined with duplex operation and good support can provide the water needed.

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