AH/LabWater™-1.10

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Standard for Laboratory Reagent-Grade Water

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This Standard is offered by the Ad Hoc Committee on Laboratory Reagent-Grade Water Standards. Comments and suggestions and encouraged and should be directed to:

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Standard for Laboratory Reagent-Grade Water

1 Scope – The AH/LabWater Standard is provided as a means for improving the control of laboratory reagent-grade water and to facilitate the accumulation and exchange of knowledge relating to the impact of water purity on laboratory applications. This Standard is not mandatory and is intended to derive its validity and value from sound, scientific reason and widespread voluntary approval. It is not intended as a guide for the preparation or analysis of reagent-grade water and laboratories are encouraged to refer to scholarly texts and articles for this information.

Note: *It is the responsibility of those using this Standard to establish that it is appropriate for the circumstances in which it is used, including but not limited to issues of safety and health.*

2 Design – The AH/LabWater Standard uses a matrix of limits for process[1] and product[2] parameters in order to specify reagent-grade water. This matrix approach permits users of this Standard to select a specification that suits the requirements of a given application. The approach also permits the future extension and maintenance of this Standard, without making the experience gained from using an older version obsolete or irrelevant.

3 Maintenance – Persons with experience in the preparation or use of reagent-grade water are encouraged to make suggestions for the extension and maintenance the AH/LabWater Standard. However, in order to maintain its character, certain tenets must be observed.

3.1 The language of this Standard must be as unambiguous as possible.

3.2 The great majority of laboratories, regardless of specialty, must be able to perform, or obtain, the testing required for their use of this Standard without incurring costs that are perceived to be excessive in relation to benefits. The importance of voluntary adherence cannot be overemphasized; only when this Standard is widely used and data honestly gathered and reported will it yield a maximum benefit.

1 Process parameters relate to the processs (system) used to produce reagent-grade water.

2 Product parameters relate to the reagent-grade water product.

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3.3 There is synergy in combining product and process specifications, because the spectrum of contamination in reagent-grade water will depend on the processes used to produce it. However, in order to maintain the general nature of this Standard, only the most definitive parameters of purification systems should be specified.

3.4 The selection of product water parameters should be consistent with the general nature of this Standard. Global product water parameters must be used whenever possible in order to achieve the greatest control for the least amount of testing and record keeping.

Figure 1

Laboratory Reagent-Grade Water Specification Matrix

Process-Rxx-TOCxxx-EUxxx

Process Limits	Product Limits		
Final-Stage of Purification	**Electrical Resistivity** MΩ-cm (referenced to 25°C)	**Total Organic Carbon** TOC µg/l	**Endotoxin*** Endotoxin Units /ml
CD Continuous Distillation	**R18** ≥18.0	**TOC010** ≤010	**EU001** ≤0.001
IX Ion-Exchange	**R17** ≥17.0	**TOC050** ≤050	**EU005** ≤0.005
Note: IX systems must produce reagent-grade water that meets or exceeds R17.	**R10** ≥10.0	**TOC100** ≤100	**EU010** ≤0.010
	R05 ≥5.0	**TOC250** ≤250	**EU035** ≤0.035*
	R01 ≥1.0	**TOC500** ≤500	**EU060** ≤0.060*
	Rxxx not determined	**TOCxxx** not determined	**EU150** ≤0.150*
			EUxxx not determined

* If these measurements are made by gel-clot testing, the third place has no practical meaning.

This figure is part of the AH/LabWater Standard and is not to be used without reference to the entire Standard, which includes essential discussion and detail – (www.high-q.com/standards.html)

3 The Specification Matrix – The matrix of limits used by the AH/LabWater Standard to specify reagent-grade water is provided in Figure 1.

3.1 The Final-Stage Process Parameter – The final-stage process parameter has two limits: “CD”, continuous distillation, and “IX”, ion-exchange, which distinguish between processes that employ continuous distillation in a final stage and processes that use ion exchange in the final stage. These limits are discussed under *Heading 6*.

3.2 The Product Parameters – The three product parameters are: 1) Resistivity, 2) Total organic carbon concentration, and 3) Endotoxin concentration. The significance and measurement of these parameters are discussed under *Heading 7*. The limits for each of the three parameters are listed in Figure 1.

3.3 Alphanumeric String Conventions – All of the reagent-grade water specifications that can be created from the matrix in Figure 1 can be described as short, readable, alphanumeric strings. The convention of this Standard is that terms are ordered as follows: Process – Resistivity – TOC – Endotoxin. And a term for each of the parameters must be included in any specification, even if a parameter is not measured. When a product parameter is not measured in a specification, “xxx” replaces the numeric component of the parameter term.

Examples of Alphanumeric String Specifications:

3.3.1 The string, “CD-Rxx-TOCxxx-EUxxx”, defines Process “CD” water for which no product parameters are measured.

3.3.2 The string, “CD-R10-TOC050-EU001”, defines Process “CD” water with a resistivity of ^{3}10 MW-cm (referenced to 25° C), a TOC of £50 μg/l, and an endotoxin level of £0.001 EU/ml.

3.3.3 The string, “CD-R10-TOCxxx-EU005", defines Process “CD” water with a resistivity of ^{3}10 MW-cm (referenced to 25° C) and an endotoxin level of £0.005 EU/ml. The TOC level is not measured.

3.3.4 The string, “IX-R17-TOC050-EU050", defines Process “IX” water with a resistivity of ^{3}17 MW-cm (referenced to 25° C), a TOC of £50 μg/l, and an endotoxin level of £0.05 EU/ml.

3.3.5 The string, “IX-R17-TOC250-EUxxx", defines Process “IX” water with a resistivity of ^{3}17 MW-cm (referenced to 25° C) and a TOC of £250 μg/l. The endotoxin level is not measured.

4 Adherence – The product parameters for reagent-grade water specified by the AH/LabWater Standard must be measured frequently enough – consistent with risk and practicality – to prevent purity variations from impacting the accuracy of measurements, reliability of procedures, or quality of results. It might be impractical to monitor each of the parameters intensively when a measurement or experiment can be repeated without significant inconvenience or expense should the product water fail to meet its specification in after-the-fact testing. On the other hand, it would be necessary to monitor intensively when the water is used for time-dependent, critical testing that cannot be repeated. Data should be recorded so trends can be used to anticipate maintenance.

5 Pretreatment – For the purposes of the AH/LabWater Standard, all stages of a water purification system that precede the defined (limited) final stage are considered to be *pretreatment*. If a “CD” or “IX” stage of a purification cannot achieve the desired product water purity without pretreatment, the effectiveness and stability of the pretreatment components must be considered in order to assess how frequently product parameters need to be measured to assure an acceptable level of control.

5.1 Undetected Exhaustion/Failure – The possibility of undetected exhaustion, or failure, of any component of a reagent-grade water purification system must be minimized through intelligent system design and monitoring that is consistent with risk.

5.1.1 Monitoring the performance of the discrete technologies in a system can be as important as determining that the product water meets its specification.

5.1.1.1 Resistivity is a sensitive indicator of ion exchange bed performance provided the resistivity is on the order of 17 MW-cm or higher. However, discrete technologies that follow ion exchange beds, such as nanofiltration and ozone or UV treatment, can lower the resistivity of the water stream sufficiently so that measuring product water resistivity becomes an insensitive indicator of upstream ion exchange bed performance.

5.1.1.2 The performance of a reverse osmosis (RO) membrane can be effectively monitored by tracking the percent rejection, essentially input water resistivity over RO water resistivity. If a purification system uses ion exchange following RO, measuring only product water resistivity will give no indication of how well the RO membrane is performing.

5.1.2 Passive redundancy can add a margin of safety when components of a purification system are prone to abrupt, or difficult to monitor, exhaustion. Thus, dividing a single ion exchange bed into two equal sections that are connected in series provides passive redundancy; no controls are necessary in order for the second section to take over as the first section begins to exhaust. When the first section exhausts to a point that is easily determined with a simple, low-cost resistivity monitor or meter, this section is removed and replaced with the second section. And a new, rinsed section is installed in the second section position. (See 5.3.3)

5.2 Microorganisms – Microorganisms are difficult contaminants to remove from water.

5.2.1 In theory, filters can be designed to block the passage of essentially all live microorganisms; however, in practice, the concentration of live microorganisms downstream of a microbiological filter can be as high, or higher, than the upstream concentration, simply because microorganisms multiply. Maintaining sterility in a water purification system is not a trivial matter. Microorganisms tend to be smaller when they grow in purified water and they can "grow through" filter pores that are smaller than they are.

5.2.2 In the absence of continuous, harsh biocidal conditions, microorganisms will grow on the surfaces of water purification systems and form biofilms. The more surface area and the greater the bioavailability of nutrients, the greater their numbers. Microorganisms flourish in beds of activated carbon and ion exchange resins and in filters, including membrane filters (i.e., reverse osmosis membranes, nano filters, etc.). Microorganisms can grow successfully and form biofilms even when nutrient concentrations are very dilute. Biofilms enable microorganisms to sequester nutrients and protect the microorganisms from periodic applications of biocides. Sloughing biofilm, free-floating microorganisms, and byproducts of microorganism growth and metabolism are potential contaminants of reagent-grade water.

5.2.3 The use of oxidants to kill microorganisms and break down organics may cause an increase in the downstream concentration of microorganisms due to an increase in the bioavailability of nutrients.

5.3 Exchange and Sorption Beds – Ion exchange and sorption materials must be highly porous and have extremely large surface areas in order to function effectively. However, these features also make the materials an ideal breeding place for microorganisms and likely to release *fines* and soluble components into the water stream. Fine particles will be present in beds of new ion exchange and sorption materials, but they also develop as the result of mechanical forces (e.g., water hammer, rapid changes in water flow, swelling during regeneration, etc.). Soluble components can be residuals from the production of the bed materials; breakdown products resulting from use; or in the case of non-virgin bed materials, contaminants accumulated during previous usage. For all these reasons, bed volumes should be kept as small as reasonably possible.

Note: Activated carbon is available in molded forms that shed fewer fines.

5.3.1 Exchange and sorption materials are available in many varieties and grades. Changing from one variety, grade, or batch to another can impact the purity of the product water significantly.

5.3.1.1 Strongly acidic exchange resins are typically polysulfonic acid derivatives of polystyrene that is cross linked with divinylbenzene. Strongly basic exchange resins are typically benzyltrimethyl ammonium hydroxide derivatives of divinylbenzene that is used to cross link polystyrene. However, by varying functional groups, cross linking, polymer backbone, bead size etc. manufacturers produce a great variety of resins, which are subsequently selected and pretreated into many different grades.

5.3.1.2 Activated carbons are made from coconut shells, walnut shells, bone char, lignite etc. and their

performance will vary significantly, depending on the type and source of the raw materials, the manufacturer, and even the batch.

5.3.2 Assuming a bed is not exhausted, contaminants in the output water are present because the bed cannot remove them. Therefore, a high percentage of the fines, soluble components, microorganisms, and by-products of microorganism growth and metabolism that are contributed by one bed will pass right though a second bed of the same type. Thus, the value of using high-grade bed materials in the final stages of a purification sequence can be largely negated by upstream beds of lower quality.

Important: It is a common practice to pretreat the water feeding point-of-use systems that use expensive, high-grade, activated carbon and ion exchange resins with relatively large, inexpensive, commercial-grade beds of similar materials in order to reduce operating costs. However, such pretreatment adds contaminants to the water that the point-of-use systems may not remove.

5.3.3 Bed materials shed and bleed considerably more contamination when they are first installed than they do under steady-state conditions. Even high-grade exchange and sorption beds can require fifty (50) bed volumes, perhaps more, of rinse in order to reach steady-state levels.

5.3.4 Batch Regeneration – Most ion exchange and sorption beds are operated in batch mode; the bed is used for a period of time and then replaced with a new, or regenerated, bed.

5.3.4.1 Exchange and sorption beds function in a quasi-chromatographic fashion when they are operated in batch mode. As these beds begin to exhaust, they do not simply become slightly less efficient – they release concentrated pulses of contaminants they accumulated out of the water stream from the time they were first placed in service. Strongly bound contaminants displace weakly bound contaminants.

5.3.4.1.1 The first pulses of contaminants to be displaced from exhausting ion exchange beds will be weakly ionized substances that will have little effect on the resistivity of the product water, precisely because they are weakly ionized. (See 7.2.1)

5.3.4.1.2 Pulses of weakly sorbed organic contaminants will be displaced from beds of activated carbon by more strongly sorbed contaminants as the beds exhaust. However, activated carbon beds will also release pulses of previously bound contaminants as the result of abrupt changes in the physical or chemical nature of the input water. Step changes of the temperature, flow rate, concentration of ions and surface active contaminants etc. can cause the release of a pulse of contaminants. And such changes are not uncommon in water purification systems. When a water softener switches on and off line with each cycle of regeneration, the concentration of ions changes dramatically.

5.3.4.2 If a bed of exchange resin or activated carbon is regenerated off-site and blended with beds from many locations during bulk regeneration, the possibility of unexpected, possibly exotic, contamination must be taken into account.

5.3.5 Upflow vs. Downflow – Exchange resin beads are only slightly more dense than water and flowing water through an uncompressed bed of resin beads in an upward direction will fluidize the bed, causing a dramatic loss of bed efficiency. Upflow is contraindicated, unless a bed can be compressed to prevent fluidization. Packing beds tightly when they are loaded is no solution, because exchange resins shrink significantly as they exchange H^+ and OH^- ions for other ions in the water stream. Flowing water through an exchange bed in a downward direction eliminates the issue of fluidization, but raises the issue of gas blocking. Water that has been pressurized is likely to form bubbles when its pressure is reduced and it comes in contact with a nucleating surface. Under downflow conditions, the bubbles will accumulate and displace water from the top of a bed. Unless means are provided for bleeding away trapped gas, water flowing through the drained portion of a bed will follow channels and the efficiency of the bed will decline sharply.

5.3.6 Continuous Regeneration – The ion exchange beds in electro-deionization (EDI) systems are regenerated continuously, so they do not exhaust in the manner of ion exchange beds that are operated in batch mode. However, EDI resin beds are prone to organic and microbial fouling because they remain in place for relatively long periods of time and cannot be purged of organics, back washed, and sanitized as effectively as batch regenerated resins.

5.3.6.1 EDI uses DC voltage to create a potential gradient that splits water into sufficient concentrations of H^+ and OH^- ions to continuously regenerate the ion exchange resins and cause the migration of contaminant ions from the purified water stream into a waste water stream. However, the potential gradient does

not eliminate fouling or the growth of microorganisms.

5.3.6.2 Water entering an EDI stage must not have such a high concentration of ions (i.e., dissolved salts) as to prevent the efficient splitting of water into H^+ and OH^-. For this reason, EDI is typically combined with reverse osmosis pretreatment, which also reduces organic and microbial fouling.

5.4 Ozone and UV – Ozone is particularly effective at killing microbial cells by damging their walls. UV (255 nm) damages the DNA and RNA of microorganisms. A large percentage of microbial cells will not be killed, but will be prevented from replicating. Microbial cells that cannot replicate may be present in large numbers, but they will be invisible to a plate count (See 7.3.4) Ozone and radicals generated by short wave length UV (185 nm) also oxidize organics. Ozone or UV treatment will reduce the resistivity of the treated water. Ions are produced as impurities are oxidized, and ozonation can also introduce other gases that form ions in solution, CO_2 for example.

5.4.1 The intensity of UV radiation actually penetrating a water stream can be reduced by a variety of factors: a) Films, including biofilms, can develop on the UV source window; b) The source lamp and protective sleeves can deteriorate (e.g., wear and tear of discharge electrodes, solarization and etching of quartz, etc.); and c) Substances in the water can absorb the UV. An UV meter, placed on the opposite side of the flow chamber from the UV source window, will provide important quality control information.

6 The Final-Stage Process Parameter –

The type, design, and performance of every component in a reagent-grade water purification system will affect the purity of the product water and how frequently product parameters must be measured. However, defining limits for every component of complete systems would be impractical in the extreme. By defining two limits for the final-stage parameter, the AH/LabWater Standard provides laboratories with a practical way of conveying meaningful information regarding the spectrum of contaminants likely to be present in a reagent-grade water. The "CD" and "IX" limits distinguish between the two most common types of laboratory water purification systems, those that use continuous distillation as a final stage and those that use ion exchange as a final stage.

6.1 Out of Definition – If this Standard is used to specify reagent-grade water, which is produced by a system with a final stage that does not match one of the final-stage process limits defined by this Standard, a note explaining the variation must be attached to the specification.

6.2 Process Limit "CD" – Process limit "CD" is defined as a final stage of purification utilizing only continuous distillation and optional UV or ozone treatment. For the purposes of this Standard, continuous distillation is defined as distillation from an essentially constant volume of water.

Caveats – A still can be: a) Exceptionally effective at removing a very broad range of water contaminants, b) Essentially inert, and c) Functionally stable over long periods of time. However, still performance is dependent on design, and designs vary widely.

6.2.1 Boiler & Boiler-Condenser Transition Stages – Contaminants that have vapor pressures lower than water must be removed from the steam before it enters the condenser stage of a still or they will not be removed.

6.2.1.1 Entrainment – Contaminants with negligible vapor pressures compared to water (e.g., most inorganic salts; particulates, including microorganisms; colloids; etc.) should be eliminated by distillation. However, undistilled boiler water can enter the condenser stage by splashing, surface entrainment, and steam entrainment.

6.2.1.1.1 Splashing – As water boils, splashing is inevitable; however, a still boiler can be designed to minimize splashing and splashes that do occur can be prevented from reaching the condenser by providing enough height above the surface of the boil-

ing water and by trapping rogue splashes in a transition stage between the boiler and condenser.

6.2.1.1.2 Surface Entrainment – The draft of steam in a still can propel a film of contaminated water along wetted surfaces from the boiler into the condenser. A still can be designed to minimize such surface entrainment by avoiding excessive steam velocities, placing of a vertical hydrophobic barrier in a transition stage between the boiler and condenser to interrupt the film of water, and condensing sufficient steam on vertical wetted surfaces to reverse the net flow of the water film.

6.2.1.1.3 Steam Entrainment – Bursting bubbles of boiler water will form mist that can be carried into a still's condenser by the draft of steam flowing from the boiler to the condenser. This stream entrainment can be controlled by designing stills with boilers that have sufficient surface area, so that boiling is not unnecessarily vigorous, and by placing an effective mist filter/trap in a transition stage between the boiler and condenser.

6.2.1.2 Boiler Water Concentration – The steam concentration of contaminants that have significant, but lower, vapor pressures relative to water will depend on their concentration in the boiler water. Therefore, means must be provided for regulating the evaporative concentration of boiler water within the design limits of the still, or the purity of the product water will be inconsistent.

6.2.2 Condenser Stage – Contaminants that have vapor pressures higher than water are removed in the condenser stage of a still and how well they are removed will depend on the design and operation of the condenser. However, the purity of the product water will also depend on the rate at which water overflows the still boiler and how effectively contamination in the ambient air (e.g., dust, volatiles, etc.) is prevented from entering the condenser.

6.2.2.1 Compound (multistage) condensers that equilibrate steam and boiling hot water in multiple, specialized compartments are necessary in order to remove contaminants efficiently. Such condensers are designed to equilibrate the product water with a compartment of steam containing low concentrations of contaminants, while concentrating the contaminants in a separate compartment of steam for efficient venting. Simple (single-stage) condensers cannot be effective, even if they operate close to the temperature of boiling water, because the distilled water product is in contact with the same compartment from which steam would be vented. Increasing the concentration of contaminants in the vent steam of a simple condenser will necessarily increase their concentration in the product water. Condensers that operate at temperatures below the boiling point of water will also be ineffective, because they cannot concentrate contaminants for effective venting.

Important: Coil and jacket heat exchanges will have cold regions (potentially near freezing during winter at northern latitudes) where the cooling water enters, unless they are fed from temperature stabilized recirculating tanks.

6.2.2.2 Contaminants with vapor pressures higher than water will evaporate from boiling water relatively more quickly than water. Therefore, their concentration in the steam that enters a still's condenser will be a function of the rate at which feed water enters the still's boiler. Overflowing water through the boiler will increase the concentrations of these contaminants in the steam entering the condenser above their concentrations in the boiler feed water. Means must be provided for regulating the rate at which water flows into the boiler of a continuous still or the purity of the product water will be inconsistent.

Important: When water overflows from the boiler of a still designed with a simple coil or water jacket condenser, the concentration of contaminants with vapor pressures higher than water is likely to be greater in the product water than the source water.

6.2.2.3 When a continuous still is not running, its condenser is not under a positive pressure of steam and particulates in the ambient air can enter through the vents. Means must be provided for sanitizing a still's condenser each time the still begins a cycle and for diverting any distilled water produced during the sanitizing period away from the storage/distribution system.

6.3 Process Limit "IX" – Process limit "IX" is defined as a final stage of purification utilizing only strong-base and strong-acid ion exchange resins that are mixed in approximately equal parts; a 0.2 micron, or finer, filter (mesh/depth or membrane), which must follow the ion exchange bed; and optional UV or ozone treatment.

Note: For the purposes of this Standard. process "IX" systems are required to produce water with a resistivity of ^{3}17 MW-cm (referenced to 25° C). This limit can be easily achieved and measured, so there is no reason to tolerate the increased levels of contamination and vari-

ability that would result from accepting a lower resistivity.

Caveats – The final-stage mixed ion-exchange bed and filter can be designed to be exceptionally effective at removing dissolved ions; particulates; and, depending on the nature of the filter, even dissolved large molecules. However, as discussed under *Heading 5*, *Pretreatment*, process “IX” systems are prone to a variety of modes of failure and exhaustion that can result in substantial, and potentially rapid, reductions of product water purity. For small-scale systems, investing in passive-redundant design is likely to be more cost-effective than investing in intensive monitoring of the product water. (See 5.1.2)

7 The Product Parameters –

The three product parameters used by the AH/LabWater Standard, resistivity, total organic carbon, and endotoxin concentration are global in nature and provide practical levels of control for a minimum amount of testing and record keeping. However, if the product water parameters are not measured accurately, reported conservatively, and interpreted correctly, the value of this Standard will be diminished.

7.1 Measurements – Water purification systems must be operating routinely and producing water at a typical rate when product parameters are measured for specification purposes. And measurements must be made, or samples obtained, at the point in a system where all purification steps have been completed and the product water exits the system. In the case of recirculating systems, measurements must be made, or samples obtained, at the last point of use on the distribution system.

7.2 Individual Parameters –

7.2.1 Resistivity – Resistivity is a sensitive means for measuring the presence of substances that carry electric current efficiently in water – well-dissociated ions that are highly mobile or carry a large number of charged groups.

7.2.1.1 Resistivity in Perspective – Substances that carry electric current efficiently in water represent only a small part of the spectrum of water contaminants and high-resistivity water can be significantly contaminated with a range of substances that interfere with laboratory applications. By contrast, traces of CO_2 will have a dramatic impact on the resistivity of water and no significant effect on most applications. Just 13 ppb of CO_2 will cause the resistivity of truly pure water to drop from 18.3 to 10 MW-cm (referenced to 25° C) and exposing the pure water briefly to clean air will cause its resistivity to drop to about 500 KW-cm (referenced to 25° C).

Important: Resistivity cannot be used to compare the relative purity of water produced by “IX” and “CD” systems, because the former exploits ion binding and the latter exploits vapor pressure, which is not ion specific.

7.2.1.2 Practicality – Laboratories should confirm the accuracy of their instruments and avoid making claims that are not statistically valid. Making accurate measurements becomes dramatically more difficult when the resistivity of a sample approaches that of pure water, 18.3 MW-cm (referenced to 25° C). The instrumentation and time required to measure resistivity with better than ±5% accuracy can cost more than many laboratories can justify, and sample referral is not an option. (See 7.2.1.4) This should be an important consideration for persons writing specifications.

7.2.1.3 Handling Samples – Resistivity testing is best performed with in-line flow cells and care should be exercised to avoid contamination. The tightness of seals and the permeability of connecting tubing are critical in order to prevent traces of CO_2 and other gases that form ions in solution from entering the water and reducing the resistivity.

7.2.1.4 Instruments – Instruments capable of making acceptable measurements in the range of 0.5 to 5.0

MW-cm may not be adequate for making measurements close to 18 MW-cm. In order to determine that a water sample has a resistivity of 18.1 and not 17.9 MW-cm (referenced to 25° C), the instrument must have an accuracy of better than ±1% over the temperature range of the water being measured. Few resistivity instruments are capable of that level of accuracy.

7.2.1.4.1 Demonstrating that a resistivity meter can perform a *resistance* measurement to the required accuracy, by substituting a precision resistor for a conductivity cell, does not prove that the meter is capable of performing a *resistivity* measurement to the required accuracy. The resistance measurement does not calibrate the cell constant, K_{cell}, for the cell(s) used to measure the resistivity of samples. And the K_{cell} value of a cell can shift significantly as the result of contamination or other factors.

Nor does calibrating a meter to a resistance confirm that electronic temperature compensation circuits are operating properly. The resistivity of water varies with temperature and as the resistivity of a sample approaches 18.3 MW-cm, the effect of temperature becomes increasingly significant and non-linear. Unless resistivity is measured at 25° C, temperature compensation will play a critical role in making accurate high-resistivity measurements.

Resistivity calibration standards must be prepared with great accuracy if they are to be used to calibrate an instrument to ±1% in the 18 MW-cm range. It is necessary to make extrapolations, because it is impractical to prepare standards above 100 KW-cm due to the effects of CO_2. Laboratories may find it preferable to send their cells to the manufacturers or to specialty groups for calibration.

7.2.2 Total Organic Carbon (TOC) – Measuring the TOC concentration of water provides valuable global information about the concentration of organics, which tend to be poorly detected by resistivity measurements.

7.2.2.1 Practicality – The cost of an instrument that is sufficiently sensitive and accurate to measure TOC in reagent-grade water is likely to exceed the cost of most laboratory water purification systems. Laboratories should consider pooling resources to support a local resource (e.g., a university lab, a commercial reference laboratory, etc.) that can provide results in a timely fashion.

7.2.2.2 Handling Samples – Sample containers must be impermeable and free of organic substances. Narrow mouth borosilicate glass containers are recommended. They should be carefully washed and then heated in an oven to 450° C (below the strain point of the glass) with an air atmosphere for approximately one hour. Screw caps should be lined with metal foil and carefully cleaned. Caps with damaged foil surfaces must not be used. Samples containing less than 50 ppb of carbon are likely to be significantly contaminated with organic carbon as the result of exposure to ambient laboratory air; containers should be closed quickly. Capped, glass containers should be placed in plastic bags to prevent particulate material from contaminating the external surfaces of the seals. Plastic bottles are not recommended, because of their permeability and the likelihood that organic contaminants from the bottles will leach into samples.

7.2.2.2.1 A representative sample of cleaned containers should be filled with water that has been specially prepared to be free of organic material and the water submitted for routine TOC analysis as confirmation that the containers are clean.

7.2.2.2.2 Many organic substances will stick to surfaces, so testing should be done to determine how rinsing the containers with sample water affects the TOC results.

7.2.2.2.3 Containers should be large enough to permit multiple measurements and, if necessary, concentration of the water sample.

7.2.2.3 Instruments – All TOC analyzers involve the oxidation of carbon to CO_2, followed by measurement of the CO_2 produced. However, the possible combinations of oxidation method and type of CO_2 detector has given rise to a considerable variety of TOC instruments. Methods of oxidation include UV radiation, UV plus persulfate, or UV plus heat (furnace). Some detectors use non-dispersive IR to measure CO_2 in a very specific, direct fashion. Resistivity detectors are not specific for CO_2. *Direct* resistivity detectors measure changes directly in the oxidation solution and are much less specific for CO_2 than *indirect* resistivity detectors, which use gas permeable membranes to eliminate, or reduce, contributions by the ionized products of non-carbon atoms (e.g., nitrogen, halides, etc.).

Depending on the design of a particular analyzer, it can be expected to be more or less sensitive to different classes of compounds, especially when the com-

pounds contain atoms other than carbon, oxygen, and hydrogen. For these reasons, it is important to determine the sensitivity profile of an analyzer for those substances that are anticipated in a water sample and ascertain whether the potential error is compatible with the accuracy required to report statistically valid results.

7.2.2.3.1 The water used to calibrate and blank TOC instruments for the analysis of reagent-grade water, must be specially prepared to be free of organic carbon and substances that might cause interference. Instruments must also be calibrated over the range of expected values.

7.2.3 Endotoxins (Bacterial Endotoxins Test – U.S. Pharmacopeia published by The U.S. Pharmacopeia Convention, Inc.) – An endotoxin parameter has been included in this Standard, because measuring for the presence of endotoxins is a quick, and relatively inexpensive means of determining whether or not Gram-negative microorganisms, or their byproducts, are contaminating the product water.

Note: The *Limulus* amebocyte lysate, LAL, cascade can be triggered by both lipopolysaccharide endotoxins, which react with cascade *Factor C*, and polysaccharide [(1,3)-beta-D-glucans], which react with cascade *Factor G*. Endotoxins are present in the cell walls of gram-negative bacteria and glucans are present in the cell walls of Eumycota (mushrooms, sac fungi, yeast, molds, etc.), certain bacteria, and green plants, including algae. Water samples that test positively with a glucan-sensitive endotoxin assay should be evaluated with a glucan-insensitive assay. It is always important to rule out cross-reactions and interferences by performing standard additions of known concentrations of endotoxin.

7.2.3.1 In purified water, microorganisms tend to be small; however, kinetic endotoxin testing (see 7.2.3.6.2) can detect the presence of fewer than 25 small cells (or their cell wall equivalents) per ml.

7.2.3.2 The presence of endotoxin, especially low levels, does not mean that a viable plate count will be positive. (See 7.3.4)

7.2.3.3 In the absence of TOC testing, endotoxin testing can serve as an indirect warning that TOC levels may be higher than acceptable, because changes in endotoxin levels typically correlate roughly with changes in TOC levels under steady-state conditions.

7.2.3.4 Practicality – Gel-clot can be performed by most laboratories; however, kinetic measurements require specialized equipment, which may cost more to purchase and operate than many laboratories may be able to justify. Referral is a practical option and considering the value of measuring the endotoxin parameter, laboratories should consider pooling resources to support a local common resource (e.g., a common university lab, a local commercial reference laboratory, etc.) that can provide results in a timely fashion.

7.2.3.5 Handling Samples – It is important to give serious consideration to the handling of samples for endotoxin testing.

7.2.3.5.1 Endotoxins are negatively charged, hydrophobic, organic molecules that are resistant to chemical and physical degradation. The terms, “sterile” and “non-pyrogenic” or “pyrogen-free,” do not define container characteristics that assure suitability for endotoxin sample collection or storage. Containers must be certified or tested to demonstrate that they are free of detectable endotoxin and interfering substances, consistent with the sensitivity of the endotoxin assay being used. Containers must also be certified or tested to demonstrate that they will not adsorb a significant amount of the endotoxin in a sample at the temperature(s) and over the period of time that the sample will be stored prior to analysis. Theoretically, the larger the sample volume the smaller the effect. Until definitive studies have been conducted, a minimum sample volume of 25 ml is suggested.

7.2.3.5.2 Refrigeration to just above freezing will reduce any breakdown of endotoxins and the growth of any microorganisms. Freezing and thawing is believed to promote adsorption to container surfaces.

7.2.3.6 Instruments – Endotoxin tests are either performed as titrations to a gelation endpoint (gel-clot) or by measurement of optical density (absorbance). The tests are enzymatic assays, so any instrumentation must provide a constant temperature to all reaction vessels during the period of incubation.

7.2.3.6.1 The gel-clot method requires only a water bath or dry-block incubator to maintain 37° C for a 60-minute incubation period. The gels are physically unstable, so the incubation equipment must minimize disturbance to the reaction tubes. Gel-clot tests are sensitive to about 0.03 EU/ml.

7.2.3.6.2 Tests based on reading optical density use reagents formulated so the turbidity that develops during the gelation reaction or the intensity of color that develops on hydrolysis of an artificial substrate can be measured with a photometer. The optical density is measured after a given period of incubation for *endpoint* methods. The rate of increase in optical density is measured for *kinetic* methods. Curves obtained by testing known concentrations of a standard reference endotoxin are used to compute endotoxin levels for unknown samples. Endpoint photometric tests are typically sensitive to 0.005 EU/ml. Kinetic tests may be as sensitive as 0.001 EU/ml when read intervals are short and individual reactions precisely timed.

7.2.3.6.3 Endpoint tests are usually done in 96-well, plastic plates that are incubated separate from the plate reader. Kinetic tests are usually done in 96-well, plastic plates, using an incubating plate reader, or in glass reaction tubes, using instruments that have been specialized for kinetic testing. In all cases, incubation temperatures must be uniform. Kinetic tests also require short read intervals (some instruments read all reaction vessels every 10 seconds) and a computer with sufficient memory to store the data and perform the necessary calculations. Instruments used to make turbidity measurements must not disrupt the fine suspensions.

7.3 Comments on Other Parameters – Certain product parameters have been so widely used by other specifications for reagent-grade water that it is important to comment on the reasons they were not included in this Standard.

7.3.1 pH – The buffer capacity of reagent-grade water with a resistivity of ^{3}1.0 MW-cm (referenced to 25° C) is so low that measuring pH has no practical significance for the great majority of applications.

7.3.2 Silica and boron – The presence of silica and boron in the product water of an "IX" process system can serve as a sensitive indicator of ion exchange bed exhaustion, because silica and boron are very weakly ionized. However, measuring resistivity and using serial, passive-redundant ion exchange beds is a more practical way for most laboratories to deal with bed exhaustion. (See 6.3.1)

Important: Statements in other standards and the literature indicating that silica interferes with a wide variety of biological assays and systems that do not specifically involve silica appear to be unsupported. Silica is so likely to be present in water produced by failing purification systems that silica has probably been incorrectly identified as the cause of interferences that were actually produced by other contaminants.

7.3.3 Na^+ and Cl^- ions – These ions are not included as product parameters, because monitoring resistivity provides global information about the concentration of ions in water and the measurement of specific ions would be inconsistent with the general nature of this Standard.

7.3.4 Viable Plate Counts – Plate counts require long incubation periods (days to weeks), which makes them impractical for most laboratories. And even when media and incubation conditions are optimized for oligotrophs, plate counts greatly under count the viable microorganisms in purified water (See UV in paragraph 5.4). If counts of viable microorganisms are necessary, epifluorescence direct counts of living cells would be a better choice of methods. However, the great majority of microorganisms present in water purification systems exist in biofilms and are not free floating. For this reason and because microbial cells can be killed, prevented from replicating, and filtered relatively easily, reagent-grade water is likely to contain a greater mass of cells that cannot replicate and microbiological byproducts (i.e., metabolites, cell fragments, etc.) than cells that can replicate.

7.3.5 Sterility – A parameter for sterility has not been included, because most laboratory applications do not require sterile water and this parameter would be inconsistent with the general nature of this Standard. Furthermore, the methods of sterilization available to most laboratories can significantly reduce the purity of reagent-grade water.

7.3.5.1 Autoclaving reagent-grade water in containers that are not tightly sealed will equilibrate the water with any volatile contaminants in the autoclave steam.

8 Storage and Distribution

Storage and Distribution – Storage and distribution are likely to contaminate reagent-grade water; however, it is important to distinguish between contaminants that adversely affect the applications for which the water is intended and those that do not. Thus, exposing reagent-grade water to air even briefly will contaminate it with CO_2 and cause a dramatic drop in its resistivity; however, traces of CO_2 have no significant impact on most applications. On the other hand, biofilms that can exist in containers or distribution systems can have an insidious affect on a wide range of applications.

8.1 Materials – The materials from which containers and distribution systems are constructed can be sources of unexpected contamination. Some highly rated materials are permeable to gases and other less volatile substances. Materials that are essentially inert under ideal conditions can be contaminated with water soluble substances, such as: a) Polymerization catalysts, monomers, or short chains of polymers; b) Plasticizers; c) Substances used to facilitate release from production molds; and d) Recycling contaminants. Imperfect plating or coating can result in unexpected contamination from substrate materials. Incomplete, or poorly passivated stainless steel can result in contamination. Materials used to join plastic and metal pipes can be sources of contaminants.

8.2 Storage Containers – Storage containers should be constructed of low permeability materials that contribute a practical minimum of contamination to the water and are not damaged by whatever sanitizing regimen is used. Vents should be closed except to balance pressure and equipped with 0.2 microns, or finer, hydrophobic filters.

8.2.1 Small containers should be sized so that each container can be emptied and potentially sanitized with ozone before being refilled. Containers that are continually topped off are likely to develop biofilms.

8.2.2 Large-scale containers (tanks) should be designed to be part of a recirculating loop and to minimize the dead volume. If the water purification system removes dissolved gases, inert gas blanketing can reduce the growth of biofilm. Argon is a better choice than nitrogen, because many organisms can fix nitrogen. Sanitizing large-scale containers must be done in a manner that is effective, does not contaminate the container, and is commensurate with the design of the distribution system. If a distribution system cannot be effectively sanitized, it may not be practical to make a great effort to sanitize the storage container.

8.3 Distribution Systems – Distribution systems should be designed to minimize stagnation effects and constructed of low permeability materials that contribute a practical minimum of contamination to the water. Also, the materials must withstand whatever sanitizing regimen is used and systems should be designed so they can be sanitized frequently.

8.4 Valves – Valves should not use moving, exposed, mechanical seals (e.g., stopcocks, ball valves, etc.), because such seals will drag contamination from the laboratory environment into the stored water. Valves should also be designed to prevent backflow.

8.4 Recirculation – Recirculation is used to minimize stagnation and to continually repurify the water in the storage container and distibution loop. Typically, the recirculation includes only the final stages (e.g.,ion exchange, UV and filtration). However, as endotoxin testing and epiflourescence direct counts of microbial cells are being more widely used, it is becoming increasingly apparent that such recirculation does not insure adequate control of microbiological contamination (See 5.4). In fact, when the recirculation does not include ultrafiltration or RO, non-replicating microbial cells and cell fragments can increase to high levels during the intervals between flushings of storage/distribution systems.

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