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Standard Guide for Bio-Applications Grade Water[1]

This standard is issued under the fixed designation D5196; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This guide is intended to describe the chemical and biological characteristics of water to be used whenever critical purity is essential to the use intended in laboratory Bio-Applications, for example, clinical, pharmaceutical, and biomedical. The importance of such a reagent is often underestimated despite the impact that it can have.

1.2 This guide is not intended to be used as a reference in preparing water for injectables. Generally, the appropriate use of this guide may include experiments involving tissue culture, chromatography, mass spectrometry, Polymerase Chain Reaction (PCR), DeoxyriboNucleic Acid (DNA) sequencing, DNA hybridization, electrophoresis, molecular biology or analyses where molecular concentrations of impurities may be important.

1.3 For all the other applications linked to an ASTM method and not bio-sensitive that require purified water, it is recommended that Specification D1193 or Test Method D5127 be consulted.

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D1125 Test Methods for Electrical Conductivity and Resistivity of Water

D1129 Terminology Relating to Water

D1193 Specification for Reagent Water

D4453 Practice for Handling of High Purity Water Samples

D5127 Guide for Ultra-Pure Water Used in the Electronics and Semiconductor Industries

D5173 Test Method for On-Line Monitoring of Carbon Compounds in Water by Chemical Oxidation, by UV Light Oxidation, by Both, or by High Temperature Combustion Followed by Gas Phase NDIR or by Electrolytic Conductivity

D5245 Practice for Cleaning Laboratory Glassware, Plasticware, and Equipment Used in Microbiological Analyses

D5391 Test Method for Electrical Conductivity and Resistivity of a Flowing High Purity Water Sample

D5542 Test Methods for Trace Anions in High Purity Water by Ion Chromatography

D5673 Test Method for Elements in Water by Inductively Coupled Plasma—Mass Spectrometry

D5996 Test Method for Measuring Anionic Contaminants in High-Purity Water by On-Line Ion Chromatography

F1094 Test Methods for Microbiological Monitoring of Water Used for Processing Electron and Microelectronic Devices by Direct Pressure Tap Sampling Valve and by the Presterilized Plastic Bag Method

3. Terminology

3.1 *Definitions*—For definitions of terms used in this guide, refer to Terminology D1129.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *endotoxins*—substances or by-products usually produced by gram negative micro-organisms that give a positive test for endotoxin in accordance with 13.2.

3.2.2 *heterotrophic bacterial counts/100 mL*— total number of viable micro-organisms present in the 100-mL sample, excluding anaerobic and microaerophilic bacteria.

3.2.3 *total organic carbon*—carbon in the form of organic compounds.

3.2.4 *water*—water complying with compositions given in Table 1.

4. Significance and Use

4.1 The purity of water is relative and is usually characterized by the limits of impurities found in the water as well as by the methods used to prepare and handle the water. Section 7 mentions the suitable methods for water preparation.

[1] This guide is under the jurisdiction of ASTM Committee D19 on Water and is the direct responsibility of Subcommittee D19.02 on Quality Systems, Specification, and Statistics.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

TABLE 1

Analytes	Maximum Concentration
Total Inorganic Analytes	1 µg/L or resistivity of 18.2 Mohm.cm @ 25°C. See Note 1
Total Organic Carbon (TOC – on-line measurement)	20 ppb
Heterotrophic bacterial counts	100 cfu/100 mL
Endotoxins (Endotoxin Unit)[A]	0.01 EU/mL
Nucleases[B]	See Note 2
Proteases[C]	See Note 2

[A] If application sensitive to endotoxins. Commercial kits and methods are available for such purpose.
[B] If applications are linked to DNA and/or RNA work.
[C] If applications involved proteins.

5. Composition

5.1 Water for Bio-Applications should be prepared (using water purification technologies) starting from water complying with the U.S. Environmental Protection Agency (EPA) National Primary Drinking Water Regulations, or from comparable regulations from the European Union or Japan. The use of such a minimum standard quality for feed water is important to decrease the risk of producing and using final purified water that would be compliant with the compositions given in Table 1 but could contain certain specific contaminants in concentrations that could affect the applications.

5.2 Recommendations for purity of water should conform to the properties and chemical limits given in Table 1; however, the suggested maximum limits and the actual impurities considered, or both, may be modified by the user based upon the intended use of the water.

5.3 Although these water types and associated grades have been defined specifically for use with ASTM Standards, they may be appropriate for other applications. It is the responsibility of the users of this standard to ensure that the selected water types or grades are suitable for their intended use.

6. Reagents

6.1 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean water types as defined in this guide.

7. Summary of Preparation Methods

7.1 The method of preparation used for the water must be designed to remove organic, inorganic, volatile, biological impurities and particulates to provide water that meets the concentration limits in Table 1. These are suggested limits, since the actual maximum levels for the individual impurities will depend on the end use for which the water is required. More restrictive limits than those suggested in Table 1 may be required by mutual consent of the parties concerned, provided a suitable test method is agreed upon.

7.2 The Bio-Applications grade water needs to be prepared from tap water complying with U.S. EPA National Primary Drinking Water regulations or comparable regulations of the European Union or Japan.

7.3 The purification of tap water shall be accomplished by a single technology or a combination of suitable purification technologies such as distillation, deionization, electrodeionization, carbon adsorption, reverse osmosis, ultrafiltration, nanofiltration, UV photo-oxidation, and/or screen membrane filtration, to meet the compositions given in Table 1.

7.4 The water purification systems containing these technologies should be constructed from materials shown to contribute to low contamination to the final product water.

7.5 Because quality assurance is key to ensure safety, efficiency and reliability, validation of the water purification installation is highly recommended (see Section 14).

8. Monitoring and Trends

8.1 The monitoring of different parameters should be performed at a frequency defined by the user to ensure with a high degree of confidence that the water quality used is always compliant with the specifications and the purpose.

8.2 Regular calibration and maintenance of the measuring instruments is the best way to ensure, with a high level of confidence, the validity of the values obtained to determine the compliance with the specifications of the water used. Trending parameters is the main reliable source of information to define maintenance schedule and to anticipate failures.

8.3 *Inorganic Analytes*—Resistivity is the most widely used parameter to monitor the overall ionic purity. According to their mobility, each ionic species will have a different effect on the resistivity. The limit of Table 1 apply to the water sampled at the point of use or, when for practical reasons and/or to avoid contamination (for example connection of an equipment after a 0.2 µm filter), as close as possible to the point of use and with a regular verification of a low impact of the purification steps and/or equipment placed downstream of the monitoring sampling point. If in-line measurements are not possible then analyses of the water produced should be conducted to determine that the total ionic concentration of all the analytes described in Table 2 does not exceed the compositions given in Table 1 (≤ 1 µg/L total). Table 2 lists common cations and anions that have an impact on the resistivity value and may have an impact on some Bio-Applications. The user should add

TABLE 2 Ionic Suggested Contaminant List

Cations	Anions
Aluminium	Chloride
Ammonium	Nitrate
Arsenic	Phosphate
Cadmium	Sulfate
Calcium	Fluoride
Chromium	
Cobalt	
Copper	
Iron	
Lead	
Magnesium	
Nickel	
Potassium	
Sodium	
Titanium	
Zinc	

any other ionic contaminants (not already indicated) to this list if the application being performed may be sensitive to those ions.

8.4 *Heterotrophic Bacterial Count*— The maximum concentrations proposed in Table 1 is given for determination by a plate-count method. If this method is selected, Test Method F1094 can be used as a reference. Such determination can be performed at a periodicity that will be defined by the user. Only viable bacteria that are able to grow on the media selected will be counted. If frequent verification with rapid results are necessary, an epifluorescence method can also be used. In this case, viable and non-viable bacteria can be counted. Therefore the maximum concentrations given in Table 1 should be adapted accordingly.

8.5 *Nucleases*—Determination of nucleases should be performed when RNA and/or DNA are used in the applications.

8.6 *Proteases*—Determination of the proteases should be performed when proteins are involved in the applications.

9. Sampling

9.1 Samplings for the test methods specified in Section 13 but also for the water that will be used for the Bio-Applications assume that great care and skill will be employed in obtaining the water samples to be tested or used. It is assumed that the operators will prevent container and airborne contamination to the best of their ability, making note of possible sources of contamination due to the sampling procedure. It is recommended that the samples be handled in accordance with Practice D4453.

9.2 Extreme care must be exercised in handling samples when making analyses. Depending on the analyte to be analyzed, experimental laboratory-ware should be selected. PFA or TFE fluorocarbon (except for fluoride analysis) or HDPE laboratory-ware should be used for ion analysis and high purity glass containers should be preferred for organic molecules analysis (TOC, volatile chlorinated hydrocarbon, phthalates, and so forth). Several samplings should be performed according to the nature of the analyte.

9.2.1 Storage of the sample may be required for the detection of metals, in which case 1 mL of re-distilled HNO_3 (1:99) should be added per litre to reduce the pH and to preserve solubility of the metals within the sample.

9.2.2 The water sample should remain in storage a minimal period of time since some analytes have a tendency to adhere to the container surface and others may leach from the container.

9.2.3 Practice D5245 should be used as a guide to clean the glassware or plasticware before microbiological analyses.

9.2.4 When endotoxin monitoring or nuclease measurements are required, special endotoxin-free and/or nuclease-free glassware is advised.

10. Recommendations for Purity

10.1 Recommendations for purity of water should conform to the properties and chemical limits given in Table 1; however the suggested maximum limits and the actual impurities considered, or both, may be modified by the user based upon the intended use of the water.

10.2 The precision of detection will depend on the purity of the reagents used, equipment employed, experience of the laboratory personnel, the sampling technique, and cleanliness of the working area.

11. Summary of Method of Storage

11.1 Storage of the final purified water should be avoided or limited to as short a time as possible. Final purified water should be protected from any external contamination, as well as contamination from the storage container used.

12. Maintenance and Calibration

12.1 Periodic preventive maintenance should be performed to ensure the long-term performance and reliability of the water purification system. Follow-up trends in the quality and performance parameters should be observed regularly to check any variations in performance of the installation and to be able to anticipate any failures.

12.2 Periodic calibration of the different measuring instruments should be performed to ensure the validity of the values obtained. Due to the difficulties in calibrating conductivity meters used for low conductivity ranges (< 1 µS/cm at 25°C), periodic verification based on comparison with externally calibrated measuring instrument may be acceptable.

12.3 The frequency of system calibration and maintenance of the system should be defined by the user depending on the importance of the water in applications, but should not be performed less than once a year.

13. Test Methods

13.1 *Total Organic Carbon (TOC)*—Test Method D5173.

13.2 *Endotoxins*—USP <85> Bacterial Endotoxins Test Method.[3]

13.3 *Heterotropic Bacterial Count*—Test Method F1094.

13.4 *Electrical Resistivity*—Test Method D1125 and Test Method D5391.

13.5 *Total Inorganic Analytes*—Test Methods D5391, D5542, D5673 and D5996. See Note 3.

NOTE 1—The resistivity value corresponds to the theoretical value of the water exempt of ions. As for a real, practical measurement, a maximum tolerance of ± 1 Mohm.cm should be accepted to take into account the accuracy of the measurement device used.

NOTE 2—If Nucleases and/or Proteases are of concerns for the applications, a purification technology that removes such contaminants should be used in the purification process (such as ultrafiltration or distillation). Such purification step should be located as close as possible to the point of use, avoiding recontamination of the water by downstream purification stages.

NOTE 3—There is no current ASTM Standard Method for the determination of all the anions and cations listed in Table 2 at the limits required by this guide. However ICP/MS and ion chromatography methods are available to measure such elements at these levels. Manufacturer's consultation can be helpful.

[3] Published in the U.S. Pharmacopeia twenty-seventh revision by The U.S. Pharmacopeial Convention, Inc.

14. Validation

14.1 Because quality assurance is the key to ensure safety, efficiency and reliability, validation is becoming increasingly important. The validation process can be divided into 4 major qualification steps:

14.2 *Design Qualification (DQ)*—The Design Qualification is carried out before the selection of water purification system is made and consists of defining the water types required depending on the applications, and defining the technology(ies) to be used, including the monitors to verify water quality. The design of the installation should also be defined according to requirements. All steps should be documented.

14.3 *Installation Qualification (IQ)*— The Installation Qualification should take place after the installation of the system and consists of verifying and documenting that the installation was performed according to the pre-determined specifications. This requires that the calibration of the various measuring instruments will be verified (if pertinent). The actual installation should be compared with an installation drawing to ensure that no future installation modifications will be able to be performed without suitable control management. Verification of the availability of all documentation required to use and maintain the system should also be done. The list of material in contact with water is recommended to ensure the compatibility of such materials with the application requirements. Documented verification of the water purification system may be performed to ensure that the installation was performed according to specifications.

14.4 *Operational Qualification (OQ)*— The Operational Qualification is performed after installation of the system and consists of ensuring that the system is operating according to the pre-determined specifications. Tests should be conducted to verify that the hydraulic, monitoring and electronic functions (including system alerts) of the systems are working according to the specifications.

14.5 *Performance Qualification (PQ)*— The Performance Qualification is carried out after the installation and operational qualification have been performed to ensure and to document that the system is performing according to the pre-determined specifications. During this qualification step, verification of the appropriateness of the specifications, defined according to the applications, and verification of the water quality produced should be conducted.

14.6 Re-qualification should be conducted on a regular time-basis and also each time components are replaced that can affect the quality or the quantity of water produced.

14.6.1 The frequency of re-qualification depends on the importance of purified water in applications but cannot exceed one year. This ensures complete annual verification of the system alerts and calibration of the measuring instrument.

14.6.2 A preventive maintenance (see Section 12) should be conducted regularly and all actions should be documented in a dedicated system logbook.

15. Keywords

15.1 bio-applications; clinical; medicinal; pharmaceutical; purified water; research