~~Designation:D 5196–91 (Reapproved 1999)~~

~~An American National Standard~~ Designation: D 5196 – 06

Standard Guide for ~~Biomedical Grade Water~~Bio-Applications Grade Water[1]

This standard is issued under the fixed designation D 5196; 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

~~1.1This guide is intended to describe the physical and chemical characteristics of water to be used whenever critical purity is essential to the use intended in clinical, pharmaceutical, biophysical, biomedical, chemical, physical research applications, or a combination of these. This guide is not intended for use in preparing water for injectables. Generally, the appropriate use of this guide may include experiments involving tissue culture, chromatography, mass spectroscopy, or analysis where molecular quantities of impurities may be important.~~

~~1.2*This standard does not purport to address all of the safety problems, 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.*~~

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 D 1193 or Test Method D 5127 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]

D 1125 Test Methods for Electrical Conductivity and Resistivity of ~~Water~~Water

D 1129 Terminology Relating to ~~Water~~[2]Water

~~D1426Test Methods for Ammonia Nitrogen in Water~~[2] 1193 Specification for Reagent Water

~~D1428Test Methods for Sodium and Potassium in Water and Water-Formed Deposits by Flame Photometry~~ 4453 Practice for Handling of Ultra-Pure Water Samples

D 5127 Guide for Ultra-Pure Water Used in the Electronics and Semiconductor Industries

~~D3919Practice for Measuring Trace Elements in Water by Graphite Furnace Atomic Absorption Spectrophotometry~~[2] 5173 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

~~D3973Test Method for Low Molecular Weight Halogenated Hydrocarbons in Water~~ 5245 Practice for Cleaning Laboratory Glassware, Plasticware, and Equipment Used in Microbiological Analyses

~~D4453Practice for Handling of Ultra-Pure Water Samples~~[2] 5391 Test Method for Electrical Conductivity and Resistivity of a Flowing High Purity Water Sample

~~D4517Test Method for Low-Level Total Silica in High Purity Water by Flameless Atomic Absorption Spectroscopy~~[4] 5542 Test Methods for Trace Anions in High Purity Water by Ion Chromatography

~~D4779Test Method for Total, Organic, and Inorganic Carbon in High Purity Water by Ultraviolet (UV), or Persulfate Oxidation,~~

---

[1] This guide is under the jurisdiction of ASTM Committee ~~D-19~~ D19 on Water and is the direct responsibility of Subcommittee D19.02 on General Specifications, Technical Resources, and Statistical Methods.

~~Current edition approved Oct. 15, 1991. Published February 1992.~~

Current edition approved April 1, 2006. Published April 2006. Originally approved in 1991. Last previous edition approved in 1999 as D 5196 – 91 (1999).

[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* ~~, Vol 11.01.~~volume information, refer to the standard's Document Summary page on the ASTM website.

or Both, and Infrared Detection[4] 5673 Test Method for Elements in Water by Inductively Coupled PlasmaMass Spectrometry
D 5996 Test Method for Measuring Anionic Contaminants in High-Purity Water by On-Line Ion Chromatography
F 1094 Test Methods for Microbiological Monitoring of Water Used for Processing Electron and Microelectronic Devices by Direct- Pressure Tap Sampling Valve and by the Pre-SterilizedPresterilized Plastic Bag MethodMethod

3. Terminology

3.1 *Definitions*—For definitions of terms used in this guide, refer to Terminology D 1129.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *endotoxins*—substances or by-products usually produced by gram negative micro-organisms whichthat give a positive test for endotoxin in accordance with 8.2413.2.

3.2.2 *heterotrophic bacterial counts/1000 mL*— total number of viable micro-organisms present in the 1000-mL100-mL sample, excluding anaerobic and microaerophilic bacteria.

3.2.3 *total organic carbon*—carbon measured after inorganic carbon response has been eliminated by one of the prescribed ASTM methods. —carbon in the form of organic compounds.

3.2.4 *water*—water prepared in accordance with this guide.

4.Significance and Use

4.1The purity of water is only relative and is usually defined by the limits of impurities found in the water as well as by the methods used to prepare and handle the water. Appendix X1 describes a method of preparation of moderate volumes of water with the highest purity practical using available equipment and techniques.

4.2The method of preparation of water described in Appendix X1 is designed to remove organic, inorganic, volatile, particulate, and biological impurities to provide water that should meet the concentration limits in —water complying with compositions given in Table 1. These are suggested limits, since the actual maxima of the individual impurities will depend upon the required end use of the water. The limits in the guide in most cases are dictated not by the desired maximum concentration of the impurities, but by the methods of analysis. More restrictive limits may be required by mutual consent of the parties concerned, provided a suitable test method is agreed upon.

4.3The guide for the storage of high purity water is very important because impurities are added to the water in proportion to the solubility, area of contact, and time of contact between the water and the materials of containment. It is important to minimize the contact time of storage and to realize that the containment materials will determine the type of contaminants. Particular emphasis must be placed upon possible contamination from the atmosphere above the water which may add biological as well as gaseous and particulate impurities.

4.4The distribution systems present a large area of contact between the water and the pipe or tubing and, therefore, must be of a very pure insoluble substance. Organic impurities, such as plasticizers, micro-organisms and their by-products, etc., are often more important considerations than inorganic impurities. Because plastic materials may vary from batch to batch, it is desirable to include limits of specific impurities as part of any installation specification.

4.5The distribution outlets or faucets must be of non-contaminating design and materials. Particular care must be given to the valve seat and joint construction. The outlet must be protected from biological contamination particularly when the use is only occasional. Ultraviolet (UV), chemical, or heat sterilization should be considered. .

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.

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

5.1*Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where

TABLE 1 Suggested Maximum Analyte Concentrations

Analytes	Maximum Concentration, µg/L
Arsenic	0. 1
Total Inorganic Analytes	1 µg/L or resistivity of 18.2 Mohm.cm @ 25°C. See Note 1
Cadmium	0.1
Total Organic Carbon (TOC – on-line measurement)	20 ppb
Chromium	1.0
Heterotrophic bacterial counts	100 cfu/100 mL
Cobalt	1.0
Copper	1.0
Fluoride	1.0
Iron	1.0
Lead	1.0
Nickel	0.1
Potassium	2.0
Silica (total)	5.0
Sodium	0.5
Titanium	1.0
Zinc	0.5
Acetate	3.0
Ammonia	1.0
Chloride	1.0
Chloroform	5.0
Formate	2.0
Nitrate	1.0
Phosphate	1.0
Phthalates	0.1
Sulfide	1.0
Sulfate	1.0
Total organic carbon (TOC)	20.0
Volatile chlorinated hydrocarbon	5.0
Endotoxins (Endotoxin Unit)	<0.03 EU/mL
Heterotropic bacterial counts	<10/1000 mL
Electrical resistivity,[A] min, MΩ-cm at 25°C:	
Electrical resistivity,[A]	0.01 EU/mL
measured at the production point not in contact with air	10.0
Nucleases[B]	See Note 2
measured from storage or distribution system in contact with air	1.0
Proteases[C]	See Note 2

[A] E If applectrical rtion sensistivie ty can bo e expressendotoxin microsie. Commens percential kits and metre chonductis are avaitylat 25°C. Thble e fondr sucth purpose.

[B] If applivicatyions aree liprnked toe DNA alnd/or RNA work.

[C] If applicathe resionst invíolved proty, ¼eins.

such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

5.2

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

6.Sampling

6.1The test methods specified in Section 8 assume that great care and skill will be employed in obtaining the water samples to be tested. 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.

6.2Extreme care must be exercised in handling samples when making analyses. Experimental laboratory-ware should be made of PFA- or TFE-fluorocarbon, and less desirably from quartz or borosilicate glass, to minimize the contamination of the water. Borosilicate glassware may leach ions at picogram-per-litre levels. The major contaminants from borosilicate glass are sodium (Na), potassium (K), boron (B), and silica (SiO_2). No detectable ions leach out of PFA- or TFE-fluorocarbon that has been properly cleaned.

6.2.1Containers should be cleaned with HNO_3 (1+4) or HCl (1+4), or both, by filling the container and allowing it to stand for a minimum of 1 h.

6.2.2The containers should be rinsed with three container volumes of a sampled water and then allowed to stand for 24 h with the same sampled water.

~~6.2.3The containers should be rinsed again twice with the sampled water before filling.~~

~~6.2.4The containers should be filled by flushing at least five volumes of the sampled water into the vessel before sealing. The seal must be of a non-contaminating material.~~

~~6.2.5Storage of the sample may be required for the detection of metals, in which case 1 mL of redistilled HNO_3(1+99) or HCl (1+99) should be added per litre to reduce the pH and to preserve solubility of the metals within the sample.~~

~~6.2.6The water sample should remain in storage a minimal length of time since some impurities have a tendency to adhere to the container surface. Endotoxins may become irreversibly stuck to glass walls, as will certain insoluble colloids.~~

~~7.Recommendations for Purity~~

~~7.1Recommendations for purity of water should conform to the properties and chemical limits given in~~ —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~~; 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.~~

~~7.2The precision of detection will depend on the purity of the reagents used, equipment employed, experience of the lab personnel, the sampling technique, and cleanliness of the working area.~~

~~7.3A suggested guide of producing, storing, and distributing water for critical purity applications is described in the Appendix. Other procedures may be employed provided the product water meets the limits in~~ . 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 ~~as modified by the specific requirements of the use.~~

~~8.Test Methods~~

~~8.1*Arsenic*—Graphite Furnace AAS, Practice D3919~~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.

~~8.2*Cadmium*—Graphite Furnace AAS,[7] Practice D3919.~~

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 ~~*Chromium*—Graphite Furnace AAS,[7] Practice D3919.~~ 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 any other ionic contaminants (not already indicated) to this list if the application being performed may be sensitive to those ions.

8.4 ~~*Cobalt*—Graphite Furnace AAS,[7] Practice D3919.~~ 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 F 1094 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.