



## Summary of Substantive Changes between the 2017 and the 2018 editions of NSF/ANSI 55 “Ultraviolet Microbiological Water Treatment Systems”

Presented to the IAPMO Standards Review Committee on June 10, 2019

**General:** The changes to this standard should not have an impact on currently listed products. The substantive changes are:

- Clarified the scope on types of treatments covered by this standard and other NSF standards (see Sections 1.2.1 and 1.2.2)
- Removed *S. cerevisiae* as a challenge organism for class B devices (see Sections 7.2.1.1, 7.2.2, 7.2.2.4.2, 7.2.2.8.2.2, 8, and Annex A).
- Added the option of series identification of systems to the label requirements of replacement components (see Section 8.3.1)

Section 1.2.1, Class A Systems: Clarified the scope on types of treatments covered by this standard and other NSF standards as follows:

### **1.2.1 Class A systems**

*Class A POE and POU systems covered by this Standard are designed to be used for treating microbiologically unsafe water, but do not reduce chemical or inert particulate contaminants. Systems covered in this Standard are designed to inactivate and/or remove microorganisms, including bacteria, viruses, *Cryptosporidium* oocysts, and *Giardia* cysts, from ~~contaminated~~ water. Systems covered by this Standard are not intended for the treatment of water that has an obvious contamination or intentional source, such as raw sewage, nor are systems intended to convert wastewater to drinking water. The systems are intended to be installed on visually clear water (not colored, cloudy, or turbid). Systems with manufacturer claims that include components or functions covered under other NSF or NSF/ANSI Standards or Criteria shall conform to the applicable requirements therein.*

*Class A systems not installed downstream of a device tested for cyst reduction / inactivation in conformance to the appropriate NSF/ANSI Standard may claim *Cryptosporidium* oocysts and *Giardia* cysts only. Class A systems installed downstream of a device tested for cyst reduction/~~inactivation~~ in conformance to ~~the appropriate~~ NSF/ANSI ~~standard~~ 53 or 58 may make a general cyst claim when used on untreated surface waters, or ground water, or both, under the direct influence of surface water.*

*NOTE — Current data support that *Cryptosporidium* oocysts and *Giardia* cysts are inactivated by UV treatment.*

### **1.2.2 Class B systems or components**

*Class B POE and POU systems covered by this Standard are designed to be used for supplemental bactericidal treatment ~~of disinfected public drinking water or other drinking water that has been tested and deemed acceptable for human consumption by the state or local health agency having jurisdiction. The system is designed to reduce~~ for the inactivation of microorganisms that may be present in drinking water (public or private) considered to be microbiologically safe and of known quality. Systems covered under this Standard are intended to inactivate normally occurring nonpathogenic nuisance microorganisms only. The Class B system is not intended for the disinfection of microbiologically unsafe*



water and may not make individual or general cyst claims. Class B systems shall not make microbiological health effects claims.

Systems covered by this Standard (Class B) are not intended to be used with water that is microbiologically unsafe or of unknown quality without adequate disinfection before or after the system. Systems with manufacturer claims that include components or functions covered under other NSF or NSF/ANSI Standards or Criteria shall conform to the applicable requirements therein.

Section 7.2, Microbiological performance: Removed *S. cerevisiae* as a challenge organism for class B devices and added T1 coliphage in the 2017 edition as follows:

**7.2 Microbiological performance**

**7.2.1 UV sensitivity of challenge organisms**

**7.2.1.1 General**

Calibration is performed to determine the UV sensitivity of the MS-2 coliphage American Type Culture Collection (ATCC)12 # 15597-BI (Class A) ~~*S. cerevisiae* ATCC # 18824 (Class B)~~, or T1 coliphage ATCC # 11303 (Class B) challenges used in the performance test methods outlined in 7.2.2.

Microbiological methods for stock culture preparation, enumeration/analysis, and storage for MS-2 coliphage ~~*S. cerevisiae*~~, and T1 coliphage shall be performed as specified in Annex A.

**7.2.2 Microbial performance testing**

Component filters or other media that may interfere with the testing of a system shall be removed or bypassed during the test.

Microbiological methods for stock culture preparation, enumerations / analysis, storage, and stock challenge concentration for challenge test for MS-2 coliphage and ~~*S. cerevisiae*~~ T1 coliphage shall be performed as specified in Annex A.

**7.2.2.1 Class A systems**

A Class A system shall deliver a UV dose at least equivalent to 40 mJ/cm<sup>2</sup> [4.0 × 10<sup>4</sup> μW-sec/cm<sup>2</sup>] at the alarm set point when the system is tested in accordance with Section 7.2.2.7 or 7.2.2.8 as applicable. The equivalence of the UV dose shall be determined by comparing the system’s inactivation of MS-2 coliphage to the inactivation obtained in accordance with Section 7.2.1.3.

**7.2.2.2 Class B systems**

A Class B system shall deliver a UV dose at least equivalent to 16 mJ/cm<sup>2</sup> [1.6 × 10<sup>4</sup> μW-sec/cm<sup>2</sup>] at a UV lamp output that is 70% of normal or at the alarm set point when the system is tested in accordance with Section 7.2.2.7 or 7.2.2.8 as applicable. The equivalence of the UV dose shall be determined by comparing the system’s inactivation of ~~*S. cerevisiae*~~ T1 coliphage cells to the inactivation obtained in accordance with Section 7.2.1.3.

**7.2.2.4.2 Challenge organism**

The appropriate organism shall be added to the above water:

MS-2 coliphage ATCC # 15597-B	5 × 10 <sup>4</sup> to 5 × 10 <sup>5</sup> PFU/mL
<del><i>S. cerevisiae</i></del> <del>ATCC # 18824</del> <u>T1 coliphage</u> <u>ATCC # 11303</u>	5 × 10 <sup>4</sup> to 5 × 10 <sup>5</sup> CFU/mL



#### **7.2.2.8.2.2 Class B systems**

For Class B systems or components, the geometric mean of all ~~S. cerevisiae~~ T1 coliphage cell counts on influent samples minus the geometric mean of counts on all effluent samples shall demonstrate a log reduction equivalent to or greater than the reduction caused by a dose of 16 mJ/cm<sup>2</sup> [ $1.6 \times 10^4 \mu\text{W}\text{-sec/cm}^2$ ] as calibrated in Section 7.2.2.

Section 8, Instructions and Information: Removed *S. cerevisiae* as a challenge organism for class B devices as follows:

#### **8 Instructions and information**

*Class A systems not installed downstream of a device tested for cyst reduction / inactivation in conformance to the appropriate NSF/ANSI Standard may claim reduction of Cryptosporidium oocysts and Giardia cysts only. Class A systems installed downstream of a device tested for cyst reduction / inactivation in conformance to the appropriate NSF/ANSI Standard may make a general cyst claim when used on untreated surface waters, or groundwater, or both, under the direct influence of surface water. Class B systems may not make individual or general cyst claims. The units evaluated in this Standard shall not make claims of reduction or inactivation of MS-2 coliphage ~~S. cerevisiae~~ or T1 coliphage.*

Section 8.3, Replacement components: Added the option of series identification of systems to the label requirements of replacement components as follows:

#### **8.3 Replacement components**

**8.3.1** *The packaging of replacement components shall be labeled with the following information:*

- *model number and name of component;*
- *model number or series identification of system(s) in which the component is to be used; and*
- *name and address of manufacturer.*

Annex A, Ultraviolet water treatment systems microbial reduction: Removed *S. cerevisiae* as a challenge organism for class B devices as follows:

#### **A.1 Summary**

*MS-2 phage ~~S. cerevisiae~~ and T1 coliphage are used as biological surrogates to determine the average UV dose output of UV water treatment systems. The methods that are used for suspension preparation, titration, and analysis of the challenge organisms for use in the sensitivity calibration and testing are presented in this Annex.*

#### **A.3 Microorganisms**

*All organisms shall be obtained from ATCC.*

- ~~*Saccharomyces cerevisiae (ATCC # 18824);*~~
- *MS-2 coliphage (ATCC # 15597-BI);*
- *Escherichia coli (ATCC # 15597) host strain for MS-2;*
- *T1 coliphage (ATCC # 11303-B1); and*
- *Escherichia coli (ATCC # 11303) host strain for T1.*



**A.7.1 Formula for YM medium to be used when *S. cerevisiae* is chosen for microbiological agent**

**A.7.1.1 YM medium nutrient broth**

<b>Ingredient</b>	<b>Amount</b>
yeast extract	3 g
malt extract	3 g
peptone	5 g
dextrose	10 g
DI water	1 L
pH	6.2 ± 0.2

YM medium nutrient broth shall be dissolved by boiling and adjusted to final pH. 10-mL aliquots shall be dispensed into 16 x 150 mm test tubes. YM medium nutrient broth shall be autoclaved at 121 ± 1 °C (250 ± 1 °F) at 15 psi for 20 min. Cooled broth shall be stored at 5 ± 3 °C (41 ± 1 °F).

**A.7.1.2 YM medium agar**

<b>Ingredient</b>	<b>Amount</b>
yeast extract	3 g
malt extract	3 g
peptone	5 g
dextrose	10 g
Bacto-agar	15 g <sup>75</sup>
DI water	1 L
pH	6.2 ± 0.2
antibiotic stock solution <sup>1</sup>	2 mL/100 mL agar
<sup>1</sup> antibiotic stock solution ingredients per 100 mL of distilled or deionized water: chlortetracycline — 0.5 g chloramphenicol — 0.5 g	

NOTE — Do not autoclave antibiotic stock solution.

YM medium agar shall be dissolved by boiling, adjusted to final pH, and autoclaved at 121 ± 1 °C (250 ± 1 °F) at 15 psi for 20 min. Autoclaved YM medium agar shall be cooled to approximately 45 to 50 °C (113 to 122 °F). Antibiotic stock solution shall be aseptically added to the YM medium agar. Tempered media shall be poured into sterile petri dishes. Agar plates shall be stored at 5 ± 3 °C (41 ± 1 °F). Plates shall be allowed to come to room temperature before use.

**A.8.1 *S. cerevisiae***

**A.8.1.1 Stock culture preparation of *S. cerevisiae***

a) Four days prior to preparing *S. cerevisiae* cells stock, 0.1 mL of freezer stock *S. cerevisiae* cells shall be transferred to a 10 mL tube of YM broth. The inoculated media shall then be incubated on a shaker water bath at 25 ± 1 °C (77 ± 1 °F) at approximately 225 rpm for 24 ± 2 h.

b) 1 mL of *S. cerevisiae* cells shall be transferred to a 10 mL tube of YM broth. The inoculated media shall then be incubated on a shaker water bath at 25 ± 1 °C (77 ± 1 °F) at approximately 225 rpm for 24 ± 2 h. Perform this initial passing of the culture for three successive passes, each 24 ± 2 h, transferring to a fresh broth tube. This step shall be repeated twice.



*c) After incubation time has elapsed on the third transfer, the *S. cerevisiae* cells shall be uniformly suspended in the tube and 5 mL shall be pipetted into each of two 2-L flasks containing 1 L of YM broth. The inoculated flasks shall be incubated on a shaker water bath at  $25 \pm 1$  °C ( $77 \pm 1$  °F) at approximately 225 rpm for  $24 \pm 2$  h.*

*d) The suspension shall be centrifuged the suspension at 2320 xg for 15 min. The supernatant shall be carefully decanted. All the cells shall be pooled from the 2-L of centrifuged suspension.*

*e) The cells shall be washed three times, using 99 mL aliquots of buffered water for each wash. The suspension shall be centrifuged at 2320 xg for 15 min between washes and the supernatant shall be carefully decanted.*

*f) After removal of the supernatant on the third wash, the cells shall be resuspend in 50 mL of buffered water.*

*g) This final suspension shall be used for UV exposures in calibration and in testing of the treatment units. These cells must be used within  $24 \pm 2$  h of harvest and stored at room temperature during this time.*

*h) *S. cerevisiae* cells shall be titrated as in A.8.1.2. The concentration of *S. cerevisiae* cells should be  $10^7$  to  $10^8$  CFU/mL. Verification of the appropriate concentration of *S. cerevisiae* cells shall be made using a hemocytometer.*

#### **A.8.1.2 Enumeration of *S. cerevisiae* cells**

*a) The viable concentration of cells shall be determined using a pour plate technique.  $10^{-5}$  to  $10^{-9}$  dilutions shall be plated in triplicate on YM agar plates. The plates shall be incubated at  $25 \pm 1$  °C ( $77 \pm 1$  °F) for 48 to 72 h prior to reading.*

*b) After incubation, plates containing 25 to 250 distinct cells shall be enumerated using a Colony Counter. The titer of the *S. cerevisiae* cell suspension shall be calculated by multiplying the number of cells obtained by the inverse of the dilution factor. Results shall be expressed as the number of cells per milliliter. The concentration of *S. cerevisiae* cells should be  $10^7$  to  $10^8$  CFU/mL.*

#### **A.10.1 Enumeration of *S. cerevisiae* cells**

*a) Serial dilutions of the influent and effluent samples ( $10^0$  to  $10^{-5}$ ) shall be made using SBDW.  $10^0$  to  $10^{-5}$  dilutions shall be plated in duplicate on YM agar plates. The plates shall be incubated at  $25 \pm 1$  °C ( $77 \pm 1$  °F) for 48 to 72 h prior to reading.*

*b) After incubation, plates containing 25 to 250 distinct cells shall be enumerated using a Colony Counter. The *S. cerevisiae* cells suspension titer shall be calculated multiplying the number of CFU obtained by the inverse of the dilution factor. Results shall be expressed as the number of CFU/mL.*

#### **A.11 Challenge verification**

After the appropriate incubation period for MS-2 Coliphage, *S. cerevisiae*, or T1 Coliphage the colonies shall be counted on all of the density determination plates. The mean number of microorganisms per milliliter for plates with 25 to 250 colonies/plaques shall be calculated. This shall verify that the challenge organism was present in the challenge test water at the optimum concentration before being added to test apparatus.