



Designation: ~~C1904 – 20~~ C1904 – 22

Standard Test Methods for Determination of the Effects of Biogenic Acidification on Concrete Antimicrobial Additives and/or Concrete Products¹

This standard is issued under the fixed designation C1904; 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 standard presents test methods for the determination of the effects of biogenic acidification on concrete products and/or efficacy of antimicrobial products to resist microbially-induced corrosion (MIC) of concrete. In these tests, the biogenic acidification is achieved by sulfur-oxidizing bacteria (SOB) that can convert elemental sulfur or thiosulfate to sulfuric acid without the use of H₂S gas.

1.2 This standard is referenced in the guideline document for MIC of concrete products. Guide C1894 provides guidance for microbially-induced corrosion of concrete products and an overview of where this test, and its options, can and should be used. This document is not intended to be a guideline document for MIC of concrete products.

1.3 This standard does not cover controlled breeding chamber tests, in which H₂S gas is produced by bacterial activity and acidification is the result of the conversion of this H₂S gas to sulfuric acid.

1.4 This standard does not cover chemical acid immersion tests, in which acidification is achieved by chemical sulfuric acid addition, not by bacterial activity. Testing protocols for chemical acid immersion are described in Test Methods C267 and C1898.

1.5 This standard does not cover tests that assess field exposure conditions or sewage pipe, concrete tank, or concrete riser network design.

1.6 This standard does not cover live trial tests where concrete coupons or other specimens are monitored in sewers.

1.7 The tests described in this standard should not be performed on concrete samples that have already been exposed to MIC conditions.

1.8 This standard does not cover concrete deterioration due to chemical sulfate attack, which is caused by the reaction of sulfate compounds that exist in wastewater with the hydration products of cement. Test methods for assessing sulfate attack are provided by Test Methods C452 and C1012/C1012M.

1.9 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

¹ These test methods are under the jurisdiction of ASTM Committee C13 on Concrete Pipe and is the direct responsibility of Subcommittee C13.03 on Determining the Effects of Biogenic Sulfuric Acid on Concrete Pipe and Structures.

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1.10 *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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.11 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 ASTM Standards:²

[C125 Terminology Relating to Concrete and Concrete Aggregates](#)
[C150/C150M Specification for Portland Cement](#)
[C192/C192M Practice for Making and Curing Concrete Test Specimens in the Laboratory](#)
[C260/C260M Specification for Air-Entraining Admixtures for Concrete](#)
[C267 Test Methods for Chemical Resistance of Mortars, Grouts, and Monolithic Surfacing and Polymer Concretes](#)
[C452 Test Method for Potential Expansion of Portland-Cement Mortars Exposed to Sulfate](#)
[C494/C494M Specification for Chemical Admixtures for Concrete](#)
[C595/C595M Specification for Blended Hydraulic Cements](#)
[C618 Specification for Coal Fly Ash and Raw or Calcined Natural Pozzolan for Use in Concrete](#)
[C822 Terminology Relating to Concrete Pipe and Related Products](#)
[C989/C989M Specification for Slag Cement for Use in Concrete and Mortars](#)
[C1012/C1012M Test Method for Length Change of Hydraulic-Cement Mortars Exposed to a Sulfate Solution](#)
[C1017/C1017M Specification for Chemical Admixtures for Use in Producing Flowing Concrete \(Withdrawn 2022\)³](#)
[C1157/C1157M Performance Specification for Hydraulic Cement](#)
[C1240 Specification for Silica Fume Used in Cementitious Mixtures](#)
[C1600/C1600M Specification for Rapid Hardening Hydraulic Cement](#)
[C1768/C1768M Practice for Accelerated Curing of Concrete Cylinders](#)
[C1894 Guide for Microbially Induced Corrosion of Concrete Products](#)
[C1898 Test Methods for Determining the Chemical Resistance of Concrete Products to Acid Attack](#)
[D1193 Specification for Reagent Water](#)

2.2 Other Standards:

[AASHTO TP 119-20 Standard Method of Test for Electrical Resistivity of a Concrete Cylinder Tested in a Uniaxial Resistance Test⁴](#)
[ATCC \(American Type Culture Collection\) Bacterial Culture Guide⁵](#)
[ATCC Microbial Media Formulations⁵](#)
[EPA 375.4 Sulfate \(Turbidimetric\)⁶](#)
[ISO 20391-1 Biotechnology—Cell counting—Part 1: General guidance on cell counting methods⁷](#)

3. Terminology

3.1 Definitions:

3.1.1 For definitions of terms used in this practice, refer to Terminology standards [C125](#) and [C822](#), and Guide [C1894](#).

4. Significance and Use

4.1 As described in Guide [C1894](#), the MIC of concrete is considered to be a three-stage process with the reduction in pH (Stage I) (for example, 12.5 > pH > 9-10), the establishment of biofilms which further lowers the pH (Stage II) (for example, 9-10 > pH > 4-6) and eventual deterioration due to biogenic acid exposure (Stage III) (for example, < ~4 pH). This standard provides standard test methods to assess the effects of different stages of MIC on concrete products and efficacy of antimicrobial products used in or on concrete.

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.

³ The last approved version of this historical standard is referenced on www.astm.org.

⁴ Available from American Association of State Highway and Transportation Officials (AASHTO), 444 N. Capitol St., NW, Suite 249, Washington, DC 20001, <http://www.transportation.org>.

⁵ Available from American Type Culture Collection (ATCC) 10801 University Boulevard Manassas, VA 20110, <http://www.atcc.org>

⁶ Available from U.S. Government Printing Office, Superintendent of Documents, 732 N. Capitol St., NW, Washington, DC 20401-0001, <http://www.access.gpo.gov>.

⁷ Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, <http://www.ansi.org>.

4.2 The tests are performed in simulated exposure solutions containing well-controlled bacterial strains that are grown in the laboratory. These tests do not require an environmental chamber and are intended to be performed as benchtop tests in biosafety level 1 laboratory conditions. These tests are suitable for simulation of the Stage II and III of MIC because the pH range of the solution can be controlled within the ranges of each stage.

4.3 This standard provides three test methods.

4.3.1 Test Method A is suitable for assessing the efficacy of antimicrobial admixtures in delaying or preventing biogenic acidification in a nutrient-rich simulated wastewater exposure solution.

4.3.2 Test Method B is suitable for assessing the effectiveness of antimicrobial admixtures in a prescribed cementitious system (Option B1) or assessing the performance of different cementitious systems (Option B2) in delaying or preventing microbially-induced corrosion of concrete in the Stage II of MIC.

4.3.3 Test Method C is suitable for assessing the suitability of cementitious systems in delaying or preventing microbially-induced corrosion of concrete in the Stage III of MIC.

4.4 The results obtained by these test methods should serve as information to be used with Guide C1894 in, but not as the sole basis for, selection of a biologically-resistant material for a particular application. No attempt has been made to incorporate into these test methods all the various factors that may affect the performance of a material when subjected to actual service.

5. Apparatus

5.1 *Analytical Balance*, accurate to at least ± 0.0001 g.

5.2 *Controlled-Temperature Laboratory or Chamber*—The laboratory or chamber shall maintain the temperature of $25 \pm 2^\circ\text{C}$.

5.3 *Autoclave*, capable of maintaining $121\text{--}123^\circ\text{C}$, to be used in sterilization and waste disposal stages (Note 1).

NOTE 1—Sterilization is important to avoid cross contamination and to dispose of waste properly. An autoclave shall be used to sterilize all media/solution and borosilicate glass media bottles used to promote bacterial growth to prevent cross-contamination by other species. Sterilization shall be performed prior to commencement of any bacterial inoculation or testing, and before waste disposal after the tests. Additional guidance on sterilization and waste disposal is provided in Section 6.

5.4 *Incubator*—capable of maintaining temperature in the range of $23\text{--}30 \pm 2^\circ\text{C}$.

5.5 *Orbital Shaker*, capable of achieving at least 80 rpm.

5.6 *Pipets and Syringes*, 1 mL, 5 mL, and 10 mL.

5.7 *Automatic Pipetor*, capable of delivering $10 \text{ mL} \pm 0.05 \text{ mL}$ liquid.

5.8 *Petri Dishes*, sterile 15 mm by 100 mm.

5.9 *Inoculating Loop*.

5.10 *Borosilicate Glass Media Bottles*, of sufficient capacity to prepare nutrient media and bacteria cultures in all test methods. These are also used to perform tests using the Test Method A. The size of the bottles should be decided depending on the size of the nutrient media and bacterial cultures to be prepared. Guidance is provided in the relevant sections.

5.11 *Containers*—The containers are used to immerse the paste or mortar specimens used in Methods B and C in the exposure media. They shall be chemically compatible with sulfuric acid. Soda-lime glasses should not be used since they are prone to decalcification under acidic conditions. Polypropylene containers are suggested. Since most of the plastic containers are not

autoclavable, other sterilization methods (for example, 70 % ethanol solution) must be used to sterilize the container. The size of the bottles should be decided depending on the size of the nutrient media and bacterial cultures to be prepared. Guidance is provided in the relevant sections regarding the size of the containers depending on the number of samples to be tested.

5.12 *Vacuum-Mixer, Bowl, and Paddle*, to ~~vacuum unit will~~ minimize entrapped air ~~formation~~ formation and dust.

5.13 *Diamond Blade Wet-Saw*, to cut cylindrical paste or mortar specimens into 2.65 ± 0.15 mm thick disks.

5.14 *Flat Surface pH Electrode*, with a precision of ± 0.02 pH.

5.15 *pH Electrode*, with a precision of ± 0.02 pH.

5.16 *Calcium Combination Ion Selective Electrode (ISE)*, which can detect 0.15 ± 0.05 mg/L to $40,000 \pm 1000$ mg/L Ca^{+2} , and can work in a pH range from 2.5 to 11.

5.17 *Multiparameter Meter*, compatible with pH electrodes and ISE.

5.18 *Loading Machine*, which is equipped with the-Ball-on-Three-Ball (B3B) test apparatus (Fig. 1) (1)⁸ and a loading system that can provide the prescribed capacity and rates of loading. It shall have been verified to have an accuracy of 1.0 %, or better, within twelve months of the time prior to use.

5.19 *Digital Caliper*, with a precision of ± 0.02 mm.

6. Sterilization and Disposal of Waste

6.1 Bacteria used in the tests covered by this standard are classified as biosafety level 1 (BSL-1) based on U.S. Public Health Safety Guidelines (2).⁹ Laboratory personnel conducting the testing must have proper training to perform standard microbiological procedures. Personal protective equipment (PPE) should be worn during testing to prevent contamination as required by BSL-1 criteria.

6.2 Sterilize all apparatus and media prior to perform any bacterial inoculation to prevent cross-contamination.

6.2.1 For autoclavable apparatus and liquid media (for example, borosilicate glass, nutrient media, water), perform sterilization by autoclaving at 121-123°C for a minimum of 15 minutes.

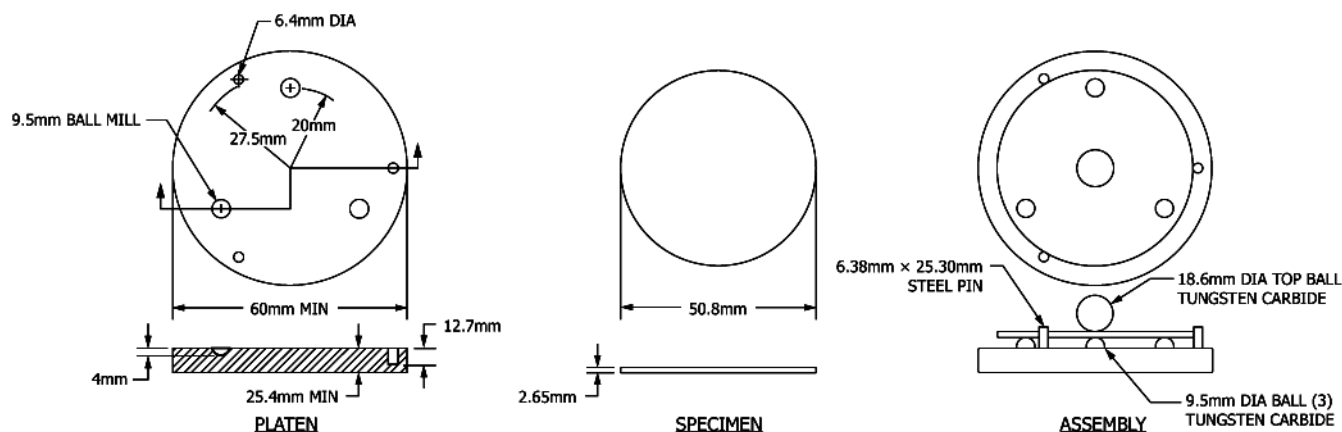


FIG. 1 Schematic Sketch of a B3B Test Apparatus

⁸ The boldface numbers in parentheses refer to a list of references at the end of this standard.

⁹ It is the responsibility of the testing facility to comply with biosafety regulations for their own country.

6.2.2 For non-autoclavable materials and apparatus, (for example, polypropylene containers, paste or mortar specimens, pH electrode, calcium ISE), use other sterilization/disinfection methods, such as rinsing with 70 % ethanol solution.

6.2.3 To prevent cross contamination of simultaneously tested cells, particularly the cells without bacterial inoculation (for example, those used on control tests without biogenic acidification), it is recommended that separate sets of apparatus be used for cells with and without bacterial cultures.

6.3 During testing, care must be taken to prevent contamination of the laboratory spaces, apparatus and supplies by proper sterilization and disinfection. After completion of the tests, sterilize all apparatus and supplies coming into contact with the bacteria media and all liquid waste, by autoclaving at 121-123°C for a minimum of 15 minutes. Sterilized waste must be disposed in accordance with related regulations mandated by related federal, state and local agencies.

7. Nutrient Media

7.1 The nutrient media (NM) is used to promote bacterial growth in all test methods.

7.1.1 In Test Method A, the NM represents the simulated wastewater solution.

7.1.2 In Test Method B, the NM is used as the exposure solution for the paste or mortar specimens.

7.1.3 In Test Method C, the NM is inoculated with the bacterial cultures to prepare a biogenic sulfuric acid solution as described in 8.2.2.

7.2 Prepare the NM by adding the following compounds to Specification D1193 Type 2 de-ionized water: 10 g/L Na₂S₂O₃, 0.25 g/L CaCl₂, 3 g/L KH₂PO₄, 3 g/L K₂HPO₄, 0.8 g/L MgCl₂ × 6H₂O, 0.1 g/L (NH₄)₂SO₄, and 5 mg/L FeSO₄ where Specification D1193 Type 2 de-ionized water is to be used as the solvent.

7.3 It is normal for the NM to have a cloudy appearance, as it will contain some undissolved solids. The NM should be stirred before it is transferred to the test cell to homogenize the suspended solids in the liquid phase.

7.4 Measured pH of the NM should be 6.55 ± 0.05.

7.5 The amount of prepared NM depends on the number of tests to be performed. The ratio between the NM volume and the total surface area of the paste or mortar specimens during tests shall be 7.0 ± 0.5 cm³/cm². This corresponds to approximately 300 mL of NM per paste or mortar specimen (disc) as described in Section 8 (Note 2).

NOTE 2—For seven specimens and solutions, 2500 mL of NM needs to be prepared.

7.6 Additional NM is required for the conditioning of the specimens as described in 9.3.

7.7 To avoid possible bacterial and/or fungal contamination during extended storage periods, the NM should be prepared as needed.

8. Bacterial Cultures and Exposure Media

8.1 The methods involve the use of bacteria that can consume elemental sulfur or thiosulfate, instead of H₂S, to acidify biogenically the exposure environment for concrete. These bacteria can be cultivated, preserved and reproduced as needed using conventional microbiological techniques such as agar plates, agar slants, and glycerol stock strains. Follow standard microbiological techniques to prepare all bacteria cultures described below.

8.2 *Test Method A*—This method uses *Halothiobacillus Neapolitanus* (ATCC 23641) and *Acidithiobacillus Thiooxidans* (ATCC 19703) as seed for biogenic sulfuric acid production. *H. Neapolitanus* is a neutrophilic sulfur-oxidizing bacteria (NSOB); and *A. Thiooxidans* is an acidophilic sulfur-oxidizing bacteria (ASOB).

8.2.1 Cultivate *H. Neapolitanus* in the ATCC Medium 290 S6 for Thiobacilli as described by the ATCC Microbial Media

Formulations guide. Cultivate *A. Thiooxidans* in the ATCC Medium 125 for Thiobacilli as described by the ATCC Microbial Media Formulations guide. The bacterial inoculation cultures are ready for the preparation of biogenic sulfuric acid when they reach their exponential growth rates as per by ATCC 290 S6 and ATCC 125. Record the average live colony forming units per milliliter (cfu/mL) of both bacterial cultures by performing a viable cell count (ISO 20391-1) (Note 3).

NOTE 3—For bacterial counting, a solid growth media that is suitable for non-fastidious bacteria (for example, TSA agar) can be used.

8.2.2 In order to test the effect of bacterial population-activity, three different acidification environments with various pH levels are created, which are designated as Severity Levels (SL) 1, 2 and 3, as described in 8.2.3, 8.2.4, and 8.2.5, respectively (Note 4).

NOTE 4—Regarding the order of testing that a lab may want to use if tests cannot be run simultaneously, if an antimicrobial product shows efficacy in SL3, it does not need to be tested for other severity levels. More information is provided in Section 10.

8.2.3 Severity Level 1 (SL1) is described as the environment with reduced bacterial population by dilution and low bacterial activity due to keeping them in nutrient-deficient media for an extended period. In these tests, fresh NM shall be acidified by actively growing bacteria until the pH drops below 3. Then, the acidified media shall be maintained in static condition in closed bottles for six weeks. During this period, the bacterial population and activity are expected to decrease due to diminishing nutrient concentration. After six weeks of waiting period, the pH of the bacterial suspension shall be measured. The suspension shall then be diluted using fresh NM to obtain several suspensions with specific pH values to determine the pH range within which the antimicrobial product is effective. Soon after the preparation of these solutions, testing should start (Note 5).

NOTE 5— It is recommended to prepare five to ten diluted solutions within the pH range from 6.5 to 2 (for example, 6.25, 6.00, 5.75, 5.10, 4.10, 3.00, and 2.00). The increased number of solutions within this range will allow more accurate determination of effective pH range of the antimicrobial product.

8.2.4 Severity Level 2 (SL2) is described as the environment with reduced bacterial population by dilution and medium bacterial activity due to keeping them in nutrient-deficient media for a period shorter than the waiting period specific for SL1. To prepare this environment, fresh NM shall be acidified with actively growing bacteria until the pH drops below 3. The acidified media shall be kept in this condition for three weeks. During this period, the bacterial population and activity is expected to decrease due to diminishing nutrient concentration. After the waiting period, the pH of the bacterial suspension shall be measured. The suspension shall be diluted by fresh NM to obtain several bacterial suspensions with specific pH values (see Note 5). Soon after the preparation of these solutions, testing should start.

8.2.5 Severity Level 3 (SL3) is the environment that is expected to have high bacterial population and activity. These tests shall be performed on media acidified by highly active bacterial cultures in fresh NM. A total of 5 % v/v of the culture of *H. Neapolitanus* and a total of 5 % v/v of the pure culture of *A. Thiooxidans* at their exponential growth rate shall be injected to fresh NM (Note 6). The pH of the environment shall not be modified through dilution of already acidified media; the bacteria are expected to reduce the pH naturally by biogenic acidification. The testing should start when target pH levels (see Note 5), as decided by the tester, are reached.

NOTE 6—As an example, 5 mL of pure bacterial culture will be injected into 100 mL of fresh NM.

8.3 *Test Method B*—This method uses *Halothiobacillus Neapolitanus* (ATCC 23641) to inoculate paste or mortar specimens. *H. Neapolitanus* is a neutrophilic sulfur-oxidizing bacteria (NSOB) and can be cultivated, preserved and reproduced as needed using conventional microbiological techniques such as agar plates, agar slants, and glycerol stock strains.

8.3.1 Cultivate the bacteria in the ATCC Medium 290 S6 for Thiobacilli as described by the ATCC Microbial Media Formulations guide. The bacterial inoculation cultures are ready for the inoculation of paste or mortar specimen when they reach their exponential growth rate. Record the average live colony forming units per milliliter (cfu/mL) of the bacterial inoculation cultures by performing a viable cell count (ISO 20391-1).

8.4 *Test Method C*—This method uses *Halothiobacillus Neapolitanus* (ATCC 23641) and *Acidithiobacillus Thiooxidans* (ATCC 19703) as seed for biogenic sulfuric acid production. *H. Neapolitanus* is a neutrophilic sulfur-oxidizing bacteria (NSOB); and *A. Thiooxidans* is an acidophilic sulfur-oxidizing bacteria (ASOB).

8.4.1 Cultivate *H. Neapolitanus* in the ATCC Medium 290 S6 for Thiobacilli as described by the ATCC Microbial Media Formulations guide. Cultivate *A. Thiooxidans* in the ATCC Medium 125 for Thiobacilli as described by the ATCC Microbial Media

Formulations guide. The bacterial inoculation cultures are ready for the preparation of biogenic sulfuric acid when they reach their exponential growth rates as per by ATCC 290 S6 and ATCC 125 (see **Note 3**). Record the average live colony forming units per milliliter (cfu/mL) of both bacterial cultures by performing a viable cell count (ISO 20391-1).

8.4.2 At their estimated exponential growth rate, inoculate the NM with 5 % (v/v) *H. Neapolitanus* and *A. Thiooxidans* (50 mL of bacteria in the growth media per 1 L of NM) to obtain biogenic sulfuric acid. Monitor pH of the inoculated media. The solution is ready for testing when the $\text{pH} = 2 \pm 0.05$.

9. Test Specimens

9.1 *Test Method A*—This test method examines the effectiveness of the antimicrobial products without the cementitious matrix. The dosage of the tested antimicrobial product could be based on manufacturer’s recommendations or decided by the tester based on other consideration.

9.2 *Test Method B*—This test method is performed on paste or mortar samples and examines either the effectiveness of cementitious materials for resisting Stage II MIC deterioration or the effectiveness of antimicrobial additives for resisting Stage II MIC deterioration in cementitious materials. This method has two options. Option B1 is used to determine the suitability of antimicrobial admixtures in delaying or preventing microbially-induced corrosion of cementitious systems in the Stage II of MIC. Option B2 is used to determine the resistance of a cementitious system in delaying or preventing microbially-induced corrosion of concrete in the Stage II of MIC.

9.2.1 In Option B1, hardened cement paste specimens will be prepared using Type I/II ordinary portland cement manufactured in compliance with Specification **C150/C150M**. Mill certificate of the cement including its oxide composition, fineness, and specific gravity shall be part of the report. The water-to-cementitious material ratio (w/cm) of the paste will be 0.42.

9.2.2 In Option B2, hardened cement paste or mortar specimens will be prepared following the required mixture proportions. The cementitious system may contain Specification **C150/C150M** portland cements (non-air entrained), Specification **C595/C595M** blended portland cements, or **C1600/C1600M** rapid hardening hydraulic cements. Additionally, supplementary cementitious materials (SCM) may be added to non-air entrained portland cements following Specification **C150/C150M**. These SCM include Specification **C618** coal fly ash and raw and calcined natural pozzolans for use in concrete, Specification **C989/C989M** slag cement for use in concrete and mortars, and **C1240** silica fume used in cementitious mixtures. Material specification reports for all cements and SCM shall be part of the reporting process. Specimens may also contain admixtures including those that are intended to increase the resistance of concrete to MIC. The type, dosage and application procedure must be specified as part of the reporting process (Specifications **C260/C260M**, **C494/C494M**, and **C1017/C1017M**). Available material specification reports for the admixtures should be provided.

9.2.3 Mixing shall be performed using a vacuum mixer with a 500 mL (or greater) bowl to minimize entrapped air formation and increase mixture consistency. The vacuum mixer bowl. If a vacuum mixer is used, it shall be operated at 400 rpm under 70 % vacuum.

9.2.4 If applicable, add the antimicrobial admixture to the required amount of Specification **D1193** Type 2 de-ionized water at the desired dosage as specified by the product’s manufacturer. Mix for 30 seconds.

9.2.5 Add the required amount of cement (and fine aggregate for mortar specimens) and mix for 90 seconds. Scrape the inner surfaces of the mixing bowl and the mixing blade using a spatula. Mix for an additional 90 seconds or until mixture appears homogeneous.

9.2.6 Cast the mixed fresh paste or mortar in plastic cylinder molds with 50.8 mm diameter and 101.6 mm height. Follow casting procedure described in Practice **C192/C192M**.

9.2.7 Seal-cure the specimens at $23 \pm 2^\circ\text{C}$ for 28 days following the procedure described in AASHTO TP 119.

9.2.8 After curing, cut the specimens into 2.65 ± 0.15 mm thick disks using a diamond blade wet saw. Only the disks obtained from the middle 60 mm part shall be used for testing. Discard the cut ends of specimens.

9.2.9 Visually inspect the specimens to discard the ones with visible cracking that was caused by the sawing process, or voids, defects, and heterogeneities that might be artifacts of poor mixing and consolidation during casting.