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.

Designation: C1904 – 22

Standard Test Methods for Determination of the Effects of Biogenic Acidification on Concrete Antimicrobial Additives and/or Concrete Products[1]

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 H_2S 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 H_2S gas is produced by bacterial activity and acidification is the result of the conversion of this H_2S 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.

1.10 *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 Surfacings 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

[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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[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.

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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 Test[4]
ATCC (American Type Culture Collection) Bacterial Culture Guide[5]
ATCC Microbial Media Formulations[5]
EPA 375.4 Sulfate (Turbidimetric)[6]
ISO 20391-1 Biotechnology—Cell counting—Part 1: General guidance on cell counting methods[7]

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.

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 ± 2°C.

5.3 *Autoclave,* capable of maintaining 121-123°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 23-30 ± 2°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 mL ± 0.05 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

[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.

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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 *Mixer, Bowl, and Paddle,* vacuum unit will minimize entrapped air 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 ± 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.

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.

[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.

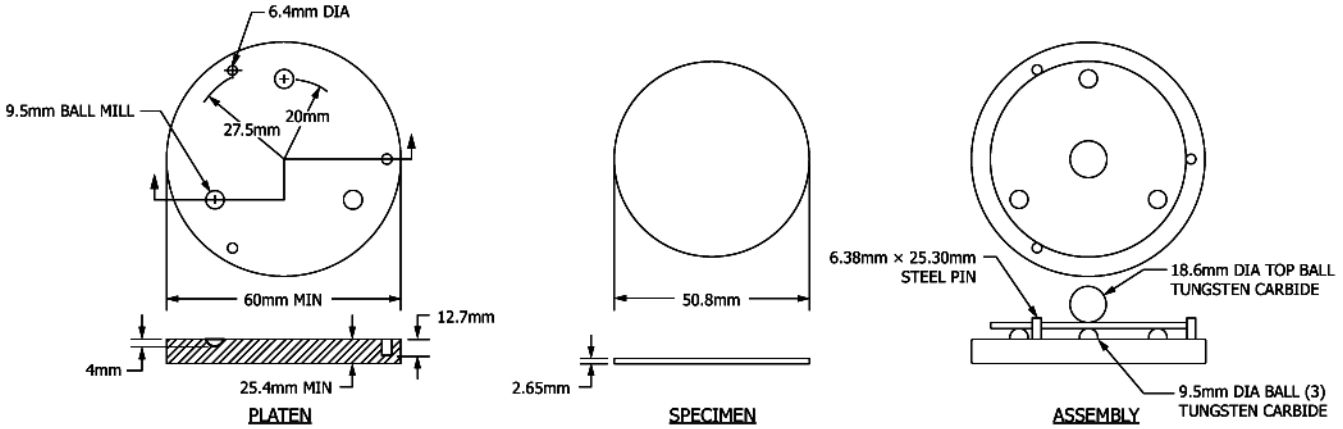

FIG. 1 Schematic Sketch of a B3B Test Apparatus

7.2 Prepare the NM by adding the following compounds to Specification D1193 Type 2 de-ionized water: 10 g/L $Na_2S_2O_3$, 0.25 g/L $CaCl_2$, 3 g/L KH_2PO_4, 3 g/L K_2HPO_4, 0.8 g/L $MgCl_2 \times 6H_2O$, 0.1 g/L $(NH_4)_2SO_4$, and 5 mg/L $FeSO_4$ 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 cm^3/cm^2. 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 H_2S, 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

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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 pH = 2 ± 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 mixer with a 500 mL (or greater) 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 ± 2°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.

9.3 *Test Method C*—Hardened cement paste or mortar specimens will be prepared following the required mixture proportions.

9.3.1 The cementitious system may contain Specification C150/C150M portland cements (non-air entrained), Specification C595/C595M blended portland cements, and 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

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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.

9.3.2 Specimens may 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.

9.3.3 Mixing shall be performed using a mixer with a 500 mL (or greater) bowl. If a vacuum mixer is used, it shall be operated at 400 rpm under 70 % vacuum.

9.3.4 If applicable, add the admixtures 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.

9.3.5 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.

9.3.6 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.

9.3.7 Cure the specimens following the desired curing protocols Practices C192/C192M and C1768/C1768M.

9.3.8 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.

9.3.9 Visually inspect the specimens to discard those 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 (Note 7).

NOTE 7—Visual detection of cracks and defects is sufficient as seven specimens will be tested and outliers will be removed. Only the disks obtained from the middle 60 mm part shall be used for testing. Discard the cut ends of specimens. Appendix X1 provides an example of a typical sample and samples that can be discarded due to cracking or defects.

10. Conditioning

10.1 Conditioning shall be done on paste specimens used for Test Method Option B1, and paste or mortar specimens used for Test Methods Option B2 and C.

10.2 Conditioning of the paste or mortar specimens involves the reduction of their pH to simulate Stage I of MIC.

10.2.1 Place the specimens in a container with tap water, either continuously or frequently refreshed until the surface pH drops below 8 ± 0.25, which may take several days. The solid-to-liquid (specimen-to-water) weight ratio shall be 1⁄30 when the water is refreshed in regular intervals.

10.2.2 Immerse the specimens in sterile NM to further reduce the surface pH and to aid the bacteria in attaching to the sample surface more easily due to absorption of the preferred nutrients on the specimen surfaces. The solid-to-liquid (specimen-to-NM) weight ratio shall be 1⁄30 during this process. The NM shall be refreshed daily for four days.

10.2.3 Measure the surface pH of the specimens on both exposed surfaces after four days of NM immersion.

10.3 For Test Method, Option B1, if applicable, apply topical antimicrobial product to surfaces of the paste specimens at the desired dosage following the instructions of the product's manufacturer (Note 8).

NOTE 8—Mass changes can be used to determine the amount of antimicrobial material applied. As degree of saturation of the specimens affects mass measurement and specimens must be dried (for example, 50°C) prior to topical antimicrobial application, the mass should be measured before and after antimicrobial application. Also, samples used for this purpose should not be used in the actual tests since drying possibly causes cracks in paste specimens.

11. Procedure

11.1 *Test Method A*—This method involves mixing the tested antimicrobial product to the exposure solution prepared for a given severity level as described in 8.2.2 – 8.2.5.

11.1.1 The testing shall start in the exposure solution prepared for SL3. If the antimicrobial product is shown to be effective in preventing or delaying bacterial acidification, other severity levels might not need to be tested.

11.1.2 If the antimicrobial product does not show effectiveness in preventing or delaying bacterial acidification in SL3, the test should be conducted in the solution prepared for SL2.

11.1.3 If the antimicrobial product does not show effectiveness in preventing or delaying bacterial acidification in SL2, the test should be conducted in the solution prepared for SL1.

11.1.4 In parallel, one cell containing only the NM, without bacterial inoculation, will be maintained as the control medium. As described in Section 6, measures will be taken to prevent cross contamination of the control media

11.1.5 Before the introduction of the antimicrobial product to the exposure solution, bacterial cell counts shall be performed (Note 9) in order to establish a quantitative bacterial population for the bacterial suspensions used in the tests. The bacterial cell counting can be performed using the viable cell count method, or other established microbiological techniques. The method of bacterial cell counting should be reported.

NOTE 9—Whenever antimicrobial admixtures are added, the cell count is to be performed immediately before the introduction of the antimicrobial product to the exposure solution to quantify the bacterial population at the time of antimicrobial addition.

11.1.6 The test involves the mixing of the tested antimicrobial product to the exposure solution and monitoring bacterial activity for 14 days for SL3, and 21 days for SL1 and SL2.

11.1.7 At least three cells will be prepared for each antibacterial product-severity level combination.

11.1.8 Each cell shall contain 150 mL of the exposure solution (Note 10).

NOTE 10—A typical dosage of the antimicrobial product is 1.5 % by mass of cementitious material, which equals to approximately 1 % by mass of cement paste when water to cement ratio (w/c) is in the range of 0.42-0.50. Since these antimicrobial agents are effective in the cement paste portion of the concrete, the dosage used in the liquid solution was accepted and used as its dosage in cement paste. Since the densities of the testing media and the antimicrobial product are almost the same, the

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dosage could also be expressed in mg/mL.

11.1.9 The cells shall be placed on a rotary shaker at 100 rpm at a controlled temperature of 25 ± 1°C (Note 11).

NOTE 11—Testing at higher temperatures can be considered; however, this shall be reported.

11.1.10 The pH of the exposure media containing the solution shall be monitored using the pH probe to monitor bacterial activity. The pH measurements shall be taken at least every 12 hours during the first week, and daily until the end of the testing.

11.1.11 The sulfate-ion concentration of the exposure media shall be monitored every 3 days (or more frequently) by taking a small amount of the exposure solution (approximately 1 mL) using a sterile syringe and analyzing it by following the EPA 375.4 procedure. Other sulfate-ion concentration determination techniques, which might enable more frequent measurements, are allowed as long as they are shown to be as or more accurate than the EPA 375.4 procedure.

11.2 *Test Method B*—This method involves immersion of inoculated and control paste or mortar specimens in NM. For each test, two tests cells are required: one for the inoculated specimens, and one for the control specimen. Each cell consists of five specimens immersed in a polypropylene container with the NM. The ratio between the NM volume and the total surface area of the specimens during tests shall be 7.0 ± 0.5 cm^3/cm^2.

11.2.1 Each side of the specimens in the inoculation cell shall be inoculated directly with 0.25 mL *H. Neapolitanus* culture, prepared as described in 7.1, using a sterile syringe and an inoculation loop. Repeated inoculations are needed to stimulate bacterial attachment during the immersion period. Since frequent inoculations would disturb the biofilm formation during handling of the specimen during the inoculation process, the inoculation frequency shall be limited to once every two days during the testing period. After each inoculation, the specimens shall be allowed to dry for approximately one hour to absorb the *H. Neapolitanus* culture before immersing into the NM.

11.2.2 The specimens in both cells shall be placed horizontally without touching each other on a polypropylene mesh serving as a specimen raiser in the cells to expose the bottom of the specimens to NM. The cells shall be placed on orbital shakers at 80 rpm at during the immersion period. The temperature of the testing room shall be maintained at 25 ± 2°C.

11.2.3 The pH of the cell containing inoculated specimens shall be maintained within the pH range of Stage II of MIC; that is, above pH = 4 ± 0.2. When the pH drops below this level, the NM will be refreshed. Regardless of the pH level, the exposure solutions will be refreshed every 8 to 12 days. During each NM refreshment, specimens shall be inoculated as well, following 11.1.1.

11.2.4 Test should continue for a minimum of three cycles (Note 12) of NM refreshment for plain samples (that is, samples without antimicrobial materials) performed due to pH drop as explained in 11.1.3.

NOTE 12—Each cycle takes approximately 8 to 12 days, as such the test can take 24 to 36 days.

11.2.5 During the immersion period, pH, free calcium-ion concentration, sulfate-ion concentration, and if elected, optical density of the exposure media shall be measured. During measurements, special attention shall be paid to prevent cross contamination of the control cell. This may require the use of separate tools and probes dedicated for the control cell.

11.2.6 The pH of the exposure media containing the specimens shall be monitored daily using the pH probe to monitor bacterial activity.

11.2.7 Free calcium-ion concentration in the exposure solution shall be monitored daily using a calcium-ion selective electrode.

11.2.8 The sulfate-ion concentration of the exposure media containing the specimens shall be monitored every three days (or more frequently) by taking a small amount of the exposure solution (approximately 1 mL) using a sterile syringe and analyzing it by following the EPA 375.4 procedure. Other sulfate-ion concentration determination techniques, which might enable more frequent measurements, are allowed as long as they are shown to be as or more accurate than the EPA 375.4 procedure.

11.2.9 (Optional) Other cell counting methods could be used to monitor the bacterial activity in the exposure solution.

11.2.10 After completion of the test, specimens shall be tested for flexural strength within 10 minutes of removal from solution using the ball-on-three-balls (B3B) test **(1)** (Note 13).

NOTE 13—In addition to the standard testing advanced characterization of paste deterioration using x-ray fluorescence, x-ray diffraction, scanning electron microscopy. Examples of this type of advanced characterization are described in References 3.

11.3 *Test Method C*—This method monitors the deterioration of paste or mortar specimens exposed to biogenic acidification representing Stage III of MIC. For each test, two tests cells are required: one containing biogenically acidified exposure media, and one containing NM for the control specimens. Each cell consists of five specimens immersed in a polypropylene container with the respective exposure solution. The ratio between the NM volume and the total surface area of the specimens during tests shall be 7.0 ± 0.5 cm^3/cm^2.

11.3.1 The specimens in both cells shall be placed horizontally without touching each other on a polypropylene mesh serving as a specimen raiser in the cells to expose the bottom of the specimens to NM. The cells shall be placed on orbital shakers at 80 rpm at during the immersion period. The temperature of the testing room shall be maintained at 25 ± 2°C.

11.3.2 The pH of the cell containing inoculated specimens shall be maintained within the pH range of Stage III of MIC; that is, below a pH of 4.0 ± 0.2. When the pH increases above this level, the exposure will be refreshed to maintain pH below 4.0 ± 0.2.

11.3.3 Test should be continued at least for 56 days.

11.3.4 During the immersion period, pH and free calcium-ion concentration of the exposure media shall be measured (Note 1).

11.3.5 The pH of the exposure media containing the specimens shall be monitored daily using the pH probe to monitor bacterial activity.

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