



Measurement of Antimicrobial Activity on Automotive HVAC Plastics, Polymer Coatings, and Other Non-Porous Surfaces

1 Introduction

Note: Nothing in this standard supercedes applicable laws and regulations.

Note: In the event of conflict between the English and domestic language, the English language shall take precedence.

1.1 Purpose. This standard is used for the qualitative and quantitative determination of antimicrobial activity on automotive Heating, Ventilation and Air Conditioning (HVAC) plastics, polymer coatings, and other non-porous surfaces. Suppressing microbial infiltration and growth can be accomplished by incorporating a nearly insoluble (sparingly soluble) antimicrobial agent(s) [e.g., silver ions (Ag^+) and/or zinc ions (Zn^{2+})], as an additive within plastics, polymer coatings, and other non-porous surfaces. This test procedure is a proficient way to evaluate antimicrobial activity and corresponding additive treatment efficacy.

1.2 Applicability. This standard is ideal, and limited to plastics, polymer coatings and other non-porous surfaces utilized in HVAC components that do not require a visible cosmetic appearance on the material surface(s). Reference Report: Establishment of Traceability and Estimation of Uncertainty in Evaluation Methods using Bacteria.

1.3 Remarks. This standard shall be used to determine the resistance to microbial growth and the associated odor emissions (metabolic waste products) from HVAC plastics, polymer coatings, and other non-porous surfaces. This standard is especially useful for designated HVAC component surfaces which are in continuous intimate contact with water during Air Conditioning (AC) operation and remain wet or moist for extended periods of time after AC operation. Reference Odor thresholds for chemicals with established occupational health standards.

2 References

Note: Only the latest approved standards are applicable unless otherwise specified.

2.1 External Standards/Specifications.

ASTM E1054[7]	EPA TO-17	ISO 12219-3	ISO/IEC 17025
ASTM E2149-13a	EU 528/2012	ISO 17025	JIS L 1902
ASTM F1980-16 Note 1	ISO 846:1997 Note 2	ISO 20743	JIS Z 2801
DIN 55531	ISO 5725-2	ISO 22196:2011(E) Note 3	PCPA 2007
EN 1040[8]	ISO 9001	ISO 27447	

Note 1: ASTM F1980–16 is used for artificial aging guidelines.

Note 2: ISO 846:1997 is used because of applicable terms.

Note 3: ISO 22196:2011(E) is used because of applicable terms.

2.2 GM Standards/Specifications.

GMW3059	GMW3259	GMW15634	GMW16151
GMW3205	GMW14444	GMW15635	GMW17082
GMW3235	GMW15600	GMW15654	

2.3 Additional References.

- Establishment of Traceability and Estimation of Uncertainty in Evaluation Methods using Bacteria, International Accreditation Japan/National Institute of Technology and Evaluation (IA Japan/NITE) Report, March 2004, available at <http://www.nite.go.jp/data/000001122.pdf>

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- Odor thresholds for chemicals with established occupational health standards may be purchased at www.aiha.org.

3 Test Preparation and Evaluation

3.1 Resources.

3.1.1 Facilities. All tests per this standard, shall be performed by a laboratory that conforms to ISO/IEC 17025 or ISO 9001 or other 3rd party accreditation that is acceptable to the GM Materials Engineer. The supplier shall submit documentation showing proof of accreditation.

Note: Some laboratories may meet GM requirements but may not be formally recognized by 3rd party accreditation.

3.1.1.1 Calibration. The test facilities and equipment shall be in good working order and shall have a valid calibration label.

3.1.1.2 Alternatives. Alternative test facilities and equipment may also be used. However, all measuring variables as specified in this standard shall be determined correctly with respect to their physical definition.

3.1.2 Equipment. This standard utilizes commonly available sampling equipment and analytical instrumentation. Unless otherwise specified, use the following apparatus and materials.

3.1.2.1 Balance, with a sensitivity of ± 0.01 mg.

3.1.2.2 Dry-heat Sterilizer, capable of maintaining the temperature at a value between 160 °C and 180 °C within ± 2 °C of the set point at equilibrium conditions.

3.1.2.3 Autoclave, capable of maintaining a temperature of 121 °C ± 2 °C and a pressure of 103 kPa ± 5 kPa.

3.1.2.4 Hotplate, with stirrer, or hot-water bath.

3.1.2.5 pH-meter, capable of measuring to ± 0.2 units.

3.1.2.6 Pipettes (sterile), with 1000 μ L tips.

3.1.2.7 Incubator, capable of maintaining the temperature within ± 1 °C of the set point at equilibrium conditions.

3.1.2.8 Vortex Mixer (if required). See ISO 22196:2011(E), section 7.6.1.

3.1.2.9 Sonicator (if required). See ISO 22196:2011(E), section 7.6.1.

3.1.2.10 Inoculating Loops, with 4 mm ring diameter, sterile.

3.1.2.11 Cover Film (which does not affect bacterial growth or absorb water), made of polyethylene, polypropylene or polyester [poly(ethylene terephthalate)]. Film that is 0.05 mm to 0.10 mm thick is recommended.

Note: Films cut from Stomacher bags are also suitable.

3.1.2.12 Screw-capped Test Tubes.

3.1.2.13 Petri Dishes (sterile), 90 mm to 100 mm in diameter.

3.1.2.14 Gauze or Absorbent Cotton.

3.1.2.15 Volumetric Flask, with a 1000 mL volume capacity.

3.1.2.16 Stoppered Erlenmeyer Flasks (or media bottles), as required for preparation of media.

3.1.3 Test Vehicle/Test Piece. This standard is used to test vehicle automotive HVAC materials that promote microbial growth. Automotive HVAC material coupon samples shall be obtained from suppliers within 7 d after production. The coupon samples manufacturing date and sampling date shall be documented. The coupon samples shall be exposed to the same preparation and manufacturing processes that the production materials would be subjected to. A minimum of twelve representative coupon samples of the material are taken for the analyses. Six (6) coupon samples shall be tested with antimicrobial additive(s), and six (6) additional control samples that do not contain the antimicrobial additive(s). The coupon samples shall be flat specimens that are 50 mm ± 2 mm $\times$ 50 mm ± 2 mm in size. Treated and untreated coating(s) applied on the sample coupon substrate shall have a depth ≥ 5 μ m ± 0.5 μ m.

Care should be taken to prevent the sample from microbial contamination, e.g., by using clean, air-tight packaging during transportation and storage and by storing packed samples under ambient conditions. The sample should not be touched with bare hands, fingers, or nails. All sample preparation shall be done with sterile and oil-free tools. The sample should be packed into aluminum composite films (e.g., Vacupack by PMG

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Incorporated or any other material per DIN 55531). In the exceptional case, the sample can be packed twice into thick silicone-free aluminum foil (cleaned with n-pentane), the edges should be folded multiple times to prevent contamination and to ensure a gastight packing. The aluminum foil packed sample should then be placed within a polyethylene bag. The bag shall be labeled with a description and part number (if available); final sample identification (cannot be changed afterwards), manufacturing date, and date of sampling. The part itself shall not be marked with any type of labeling.

Note: Samples without proper identification labels shall not be analyzed until written clarification is received. Prior to analysis, the sample should be taken from the packaging, unwrapped and stored open at $23\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$ room temperature for 2 h to prevent condensation. From manufacturing to analysis, it should be assured that the sample has been stored open at room temperature for no more than 7 d.

3.1.4 Test Time.

Calendar time: 1 d

Test time: 8 h

Coordination time: 4 h

3.1.5 Test Required Information.

3.1.5.1 Materials. Part number, drawing, and circa dimensions of the automotive material are supplied.

3.1.5.2 All Samples. Manufacturing date and sampling date must be provided.

3.1.6 Personnel/Skills. Proper training is required to operate the instrumentation within this procedure. Professional degrees in biology, biochemistry, chemistry, microbiology, or material sciences are preferred.

3.2 Preparation. Sterilization of apparatus and storage of stock cultures:

3.2.1 Dry-heat Sterilization. See ISO 22196:2011(E), section 6.1.

3.2.2 High-pressure Steam Sterilization. See ISO 22196:2011(E), section 6.2.

3.2.3 Preparation of Glassware. See ISO 22196:2011(E), section 6.3.

3.2.4 Maintenance of Stock Cultures. See ISO 22196:2011(E), section 6.4.

3.3 Conditions. See ISO 22196:2011(E), section 8.2.

Note: Test validity is defined by ISO 22196:2011(E), section 8.2.1 and repeatability and reproducibility are discussed quantitatively in ISO 22196:2011(E), Annex B.

3.3.1 Environmental Conditions. Ambient conditions $23\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$ or as specified by sample submitter.

3.3.2 Test Conditions. Deviations from the requirements of this standard shall have been agreed upon. Such requirements shall be specified on component drawings, test certificates, reports, etc.

3.3.3 Pretest Aging Protocol for Polymer Coatings. The removal of soluble organic antifungal agents, in-can preservatives and residual solvents from treated and untreated cured polymer coatings is required to prevent false positive test results for this specification. This requires a humidified thermal extraction followed by a dry thermal extraction for a specified accelerated aging time (AAT)

3.3.3.1 Humidified Thermal Extraction. Samples are to be stored in an enclosed chamber which allows the sample to come into contact with humidified air but without being submerged in water during the thermal extraction process. This chamber is to be stored within an incubator at a set accelerated aging temperature (T_{AA}) of $65\text{ }^{\circ}\text{C}$ for a minimum AAT of 14 d. See Appendix A, Figure A1. A commercially available laboratory humidified thermal extractor may substitute for the testing.

3.3.3.2 Dry Thermal Extraction. Samples are to be stored dry on a tray within a laboratory incubator at a set T_{AA} of $65\text{ }^{\circ}\text{C}$ for a minimum AAT of 30 d.

3.3.3.3 Post Aging Efficacy. If the untreated control sample results presented a false positive ($\geq 98.0\%$ efficacy, see 3.5.5) after performing the pretest aging protocol, consult with the responsible GM Materials Engineer.

3.3.3.4 Estimating AAT. See ASTM F1980-16 for artificial aging guidelines. An estimation on artificial accelerated aging is based on Equation 1 and Table 1.

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Equation 1: Estimating AAT

$$AAT = \frac{RT}{Q_{10}^{[(T_{AA} - T_{RT})/10]}}$$

Where:

AAT = Artificial aging time, in days.

RT = Desired real time, in days.

Q₁₀ = Aging factor, typically 1.8 to 2.5, with 2.0 being most common.

T_{AA} = Accelerated aging temperature, 65°C.

T_{RT} = Ambient temperature, typically 20°C to 25°C, with 23°C being most common.

Table 1: Estimating Accelerated Aging Times using Equation 1

	RT (months)	RT (days)	Q ₁₀	T _{AA} (°C)	T _{RT} (°C)	AAT (days) Note 1
Humidified	8.45	257	2	65	23	14
Dried	18.12	551	2	65	23	30
Total Note 2	26.56	808	2	65	23	44
Humidified	11.44	348	2	65	23	19
Dried	24.56	747	2	65	23	41
Total	36.00	1095	2	65	23	60
Humidified	19.10	581	2	65	23	32
Dried	40.90	1244	2	65	23	68
Total	60.00	1825	2	65	23	99

Note 1: The calculated AAT column is typically rounded up to the nearest whole day.

Note 2: Minimum AAT row requirement.

3.4 Instructions.

3.4.1 Pre-culture of Bacteria. See Section 4 and ISO 22196:2011(E), section 7.1.

3.4.2 Preparation of Test Specimens. See Section 4 and ISO 22196:2011(E), section 7.2.

3.4.3 Preparation of Test Inoculum. See Section 4 and ISO 22196:2011(E), section 7.3.

3.4.4 Inoculation of Test Specimens. See Section 4 and ISO 22196:2011(E), section 7.4.

3.4.5 Incubation of the Inoculated Test Specimens. See Section 4 and ISO 22196:2011(E), section 7.5.

3.4.6 Recovery of Bacteria from Test Specimens. See Section 4 and ISO 22196:2011(E), section 7.6.

3.4.6.1 Test Specimens immediately after Inoculation. See Section 4 and ISO 22196:2011(E), section 7.6.1.

3.4.6.2 Test Specimens after Incubation. See Section 4 and ISO 22196:2011(E), section 7.6.2.

3.4.7 Determining the Viable Bacteria Count by the Pour Plate Culture Method. See ISO 22196:2011(E), section 7.7.

3.5 Data.

3.5.1 Expression of Results.

3.5.1.1 Determination of the Number of Viable Bacteria. See ISO 22196:2011(E), section 8.1. For each test specimen, determine the number of viable bacteria recovered in accordance with Equation 2.

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Equation 2: Determination of the Number of Viable Bacteria

$$N = \frac{100 \times C \times D \times V}{A}$$

Where:

N = Number of viable bacteria recovered per square centimeter (cm²) per test specimen.

C = Average plate count for the duplicate plates.

D = Dilution factor for the plates counted.

V = Volume, in milliliters (mL), of Soybean-Casein-Digest-Lecithin-Polysorbate (SCDLP) added to the specimen.

A = Surface area, in square millimeters (mm²), of the cover film.

3.5.1.2 Determine Viable Bacteria Recovered Immediately after Inoculation from the Untreated Test Specimens. See ISO 22196:2011(E), section 8.2.2. The logarithmic value of the number of viable bacteria recovered immediately after inoculation from the untreated test specimens shall satisfy the Equation 3.

Equation 3: Logarithmic Value of the Number of Viable Bacteria

$$\frac{(L_{max} - L_{min})}{L_{mean}} \leq 0.2$$

Where:

L_{max} = Common logarithm, i.e., base 10 logarithm, of the maximum number of viable bacteria found on a specimen.

L_{min} = Common logarithm of the minimum number of viable bacteria found on a specimen.

L_{mean} = Common logarithm of the mean number of viable bacteria found on the specimens.

3.5.1.3 Determine Average Viable Bacteria Recovered Immediately after Inoculation from the Untreated Test Specimens. See ISO 22196:2011(E), section 8.2.3.

Where: $6.2 \times 10^3 \text{ cells/cm}^2 \leq L_{mean} \leq 2.5 \times 10^4 \text{ cells/cm}^2$ is satisfied.

3.5.1.4 Determine Viable Bacteria Recovered from each Untreated Test Specimen after Incubation for 24 h. See ISO 22196:2011(E), section 8.2.4.

Where: $L_{min} \geq 6.1 \times 10^1 \text{ cells/cm}^2$ is satisfied.

3.5.2 Calculation of the Antimicrobial Activity. See ISO 22196:2011(E), section 8.3. When the test is deemed valid, calculate the antimicrobial activity (R) using Equation 4, recording the result to one (1) decimal place.

Equation 4: Calculation of the Antimicrobial Activity

$$R = (U_t - U_0) - (A_t - U_0) = U_t - A_t$$

Where:

R = Antimicrobial activity, in cells/cm².

U₀ = Average of the common logarithm of the number of viable bacteria, in cells/cm², recovered from the untreated test specimens immediately after inoculation.

U_t = Average of the common logarithm of the number of viable bacteria, in cells/cm², recovered from the untreated test specimens after 24 h.

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$\bar{A}_t$ = Average of the common logarithm of the number of viable bacteria, in cells/cm², recovered from the treated test specimens after 24 h.

3.5.3 Calculate Effectiveness Ratio of the Antimicrobial Agent. The efficacy ratio value of antimicrobial activity can be used to characterize the effectiveness of an antimicrobial agent. Calculate the average efficacy ratios ($\bar{E}_0$) and ($\bar{E}_t$), using Equations 5 and 6 respectively, recording the results to three (3) decimal places.

Equation 5:

$$\text{Untreated specimen: } \bar{E}_0 = \log_{10} \left(\frac{\bar{U}_0}{\bar{U}_t} \right)$$

Equation 6:

$$\text{Treated specimen: } \bar{E}_t = \log_{10} \left(\frac{\bar{U}_0}{\bar{A}_t} \right)$$

Where:

$\bar{E}_0$ = Average untreated efficacy ratio, unitless value, recovered untreated test specimens immediately after inoculation compared to recovered treated test specimens after 24 h.

$\bar{E}_t$ = Average treated efficacy ratio, unitless value, recovered from untreated test specimens after 24 h compared to recovered treated test specimens after 24 h.

$\bar{U}_0$ = Average of the common logarithm of the number of viable bacteria, in colony forming units/cm², recovered from the untreated test specimens immediately after inoculation.

$\bar{U}_t$ = Average of the common logarithm of the number of viable bacteria, in colony forming units/cm², recovered untreated test specimens after 24 h (control).

$\bar{A}_t$ = Average of the common logarithm of the number of viable bacteria, in colony forming units/cm², recovered from the treated test specimens after 24 h.

3.5.4 Calculate Effectiveness Percentage of the Antimicrobial Agent. The efficacy percentage value of antimicrobial activity can be used to characterize the effectiveness of an antimicrobial agent. Calculate the average efficacy percentages ($\bar{P}_0$) or ($\bar{P}_t$), using Equations 7 or 8 respectively, recording the results to three (3) decimal places

Equation 7:

$$\text{Untreated specimen: } \bar{P}_0 = \left(\frac{\bar{U}_0 - \bar{U}_t}{\bar{U}_0} \right) \times 100\%$$

Equation 8:

$$\text{Treated specimen: } \bar{P}_t = \left(\frac{\bar{U}_0 - \bar{A}_t}{\bar{U}_0} \right) \times 100\%$$

Where:

$\bar{P}_0$ = Average untreated efficacy percentage value of antimicrobial activity, in percent (%), recovered from untreated test specimens after 24 h (control).

$\bar{P}_t$ = Average treated efficacy percentage value of antimicrobial activity, in percent (%), recovered from treated test specimens after 24 h.

3.5.5 Determination of Pass or Fail. The antimicrobial activity values that are used to define the overall efficacy shall be agreed upon by all interested parties. The average efficacy ratios ($\bar{E}_0$) or ($\bar{E}_t$), using Equations 5 or 6

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