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Standard Test Method for Determination of the Microbial Condition of Paint, Paint Raw Materials, and Plant Areas[1]

This standard is issued under the fixed designation D5588; 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 test method covers a procedure for the determination of the microbial condition (contamination or sterility) of raw materials used in the manufacture of paint, and the microbial condition of paint and paint manufacturing areas.

1.2 The values in SI units are to be regarded as the standard. The values given in parentheses are for information only.

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

1.4 *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. Summary of Test Method

2.1 This test method outlines procedures to (*1*) obtain samples for sterility testing from wet or dry materials and plant sites, (*2*) conduct the sterility testing on those samples to see if they are contaminated, (*3*) evaluate the degree of contamination, if any, and (*4*) provide a guide for some indication of the type of contamination present (bacterial, fungal, yeast, etc.). This test method is not designed to include all the necessary precautions to maintain the level of sterility required to provide the most accurate results. Some familiarity with microbiological techniques is recommended.

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3. Significance and Use

3.1 Spoilage of paint in the container is often related to the use of contaminated raw materials, water (particularly recycled washwater), vessels, piping, and equipment in the manufacturing plant. There is a need for a simple method to determine the presence or absence of microorganisms in plants that manufacture paints and coatings. Such a determination enables the manufacturer to establish the point of contamination (that is, raw materials or problem housekeeping areas in the plant) to help in solving the spoilage problem.

NOTE 1—Some contamination in plant areas is to be expected, since microorganisms are ubiquitous and cannot generally be eliminated practically (it is what an in-can preservative is supposed to control). Excessive levels of contamination or contaminated raw materials can exceed the capability of the preservative. If you have excessive contamination in the plant, there are methods for decontamination including steam, preservatives, bleach, etc. These should be discussed with your biocide supplier and used with care. Recovery of spoiled or contaminated products is often not feasible, so an adequate level of the appropriate biocide in conjunction with good plant housekeeping practices are essential. Your biocide supplier can also help here.

3.2 This test method may be used by persons without basic microbiological training, but some training on aseptic techniques would be recommended.

NOTE 2—The reliability of the results obtained from this test method is extremely dependent on the techniques employed. Improper techniques can result in a sterile sample appearing to be contaminated, and even worse, a contaminated sample appearing to be sterile (see also 5.1). It is recommended that you consult with your biocide supplier, raw material supplier, or an independent testing laboratory to confirm questionable results.

4. Apparatus and Materials

4.1 *Balance,* capable of weighing to 0.10 g.

4.2 *Incubator,* or other device capable of maintaining a constant temperature between 28 and 32°C.

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[1] This test method is under the jurisdiction of ASTM Committee D01 on Paint and Related Coatings, Materials, and Applications and is the direct responsibility of Subcommittee D01.28 on Biodeterioration.

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4.3 *Refrigerator.*

4.4 *Tryptic Soy Agar (TSA) Plates,*[2]pre-prepared.[3] (See Note 3).

4.5 *Potato Dextrose Agar (PDA) Plates,*[4]or *Malt Agar Plates*,[5] acidified to pH 3.5 with lactic acid, pre-prepared.

NOTE 3—If preparing plates, Tryptic Soy Agar media with TTC (triphenyltetrazolium chloride) indicator dye may also be used. In general, the TTC helps visualize contamination, but it has been reported on occasion to inhibit the growth of some bacteria. Interferences from pigments in materials being tested may make the color change difficult to see. If self-prepared plates are used with the TTC indicator, 0.01 % TTC indicator should be used and it must be added *after* autoclaving.

4.6 *Lactic Acid.*

4.7 *Sterile Swabs* in tubes, pre-prepared.

4.8 *Swab ~~tubes,~~Tubes, Culturette Tubes,* or a similar system (swab in a test tube with a transport medium)[6], all sterile, pre-prepared can be used if transport of collected samples to the laboratory testing area is required.

4.9 *Sterile Diluent* (9 mL) in tubes, pre-prepared (0.85 % saline or other suitable diluent). These can be prepared from sterile tubes and sterile saline solution then stored in a refrigerator.

4.10 *Laminar Flow Hood, Sterile Room,* or at least a laboratory testing area that is relatively clean, free of blowing dust and dirt, etc., which can be used for streaking plates.

4.11 *Antiseptic Solution,* to help maintain sterility of testing area surfaces (4.10) (For example, 70 % ethanol solution.).

4.12 *Plastic or Rubber Laboratory Gloves,* optional, sterilized.

4.13 *Facial Mask,* optional.

4.14 *Sterile Spatulas or Sterile Tongue Depressors,* 150-mm, (6-in.) individually wrapped.

4.15 *Plastic Bag,*[7]sterile.

5. General Sampling Guidelines

5.1 Take all reasonable precautions to avoid microbial contamination while obtaining samples. You may choose to wear a facial mask and sterilized gloves. (**Warning**—Do not touch the swab anywhere near the cotton tip, or near parts of the swab which could be immersed in the test sample. Microorganisms from the skin, clothing, and even air if exposed too long, can contaminate the sample. If the swab has a cap, do not touch any part of the swab except that cap. Confirm suspicious results with additional testing.)

5.2 Use a new sterile swab, tongue depressor or spatula for each sample. Do not reuse any sampling devices. If using gloves, dispose after use.

5.3 When taking samples, be sure to minimize the time sterile items are exposed to the air to avoid false contamination results.

5.4 Liquid materials may be sampled as outlined in Section 6. Alternately, a sterilized container may be used to transport the liquid sample to the sterile testing area. Be sure that no non-sterile items contact the liquid sample during sampling, handling, and movement to the testing area (for example, use sterile pipet, etc. for transfer of material to container, etc.).

5.5 Dry materials may be sampled as in 6.3 or 9.1. To sample unopened, dry raw materials in bags, wipe a large area of the outside of the bag clean with a clean rag or paper towel. Using a clean knife, cut open the bag within the cleaned area. Sample as in 9.1, or using a sterile tongue depressor or sterile spatula, scoop 10 to 15 g into a sterile plastic bag,[7] close and seal bag for transport to sterile testing area.

NOTE 4—To decrease the chances of inadvertent contamination, a suggestion would be to carefully wipe the area of the bag to be cut, and the knife used for cutting it, with isopropyl alcohol. **Warning**: Exercise care to avoid skin contact, since the isopropyl alcohol could carry hazardous materials through the skin. Also, avoid excess alcohol that could affect test results.

5.6 When testing open containers of raw materials, vats, drums, etc., there is no need to sterile equipment surfaces (see Section 6). However, be aware that any contamination observed may have been introduced after opening. Samples taken from equipment surfaces that show contamination do not necessarily mean that the material contained or being manufactured inside is also contaminated.

[2] Please note that Tryptic Soy and Trypticase Soy are names used interchangeably. Pre-prepared TSA plates, BBL# 21185, are available from various microbiological supply companies.

[3] Agar plates (media) may be purchased pre-prepared using the indicated Difco or BBL number from microbiological supply companies, or both. Media may also be prepared from the formulations given in the *Difco Manual* (Difco Laboratories, Detroit, MI) or from appropriate dehydrated media using standard microbiological techniques.

[4] Pre-prepared plates available are Difco # 4360-22-0, or BBL # 96272. These pre-prepared plates are not acidified to pH 3.5, but may be used (see also Footnote 3).

[5] Pre-prepared plates available are Difco # 4265-22-6. These are not acidified, but may be used (see also Footnote 3).

[6] Available from microbiological supply companies. Swab tubes or culturette tubes 9345 with Amies medium were used.

[7] Sterile plastic packs are available from microbiological supply companies.

6. Sampling Procedure for Plant Areas

6.1 Establish a protocol for surveying probable areas of contamination. Make sure that such areas include pipes and hoses, especially if left with water standing, any storage and mixing vessels, pumps, drains, sumps, etc. Because recycled washwater is particularly susceptible to contamination, be sure to include it.

6.2 Sampling is best carried out when the area to be tested is wet. In wet areas, the swab is dipped into or wiped on the area (see Note 3), and then returned to a sterile tube (with or without transport media). This swab is then used for testing as described in Section 8 (see also Section 7).

6.3 Sampling dry areas provides information that is less conclusive, but can be carried out by swabbing the dry area with a sterile swab that has previously been dipped into sterile diluent. This swab is then used as described in Section 8.

7. Testing Transported Samples

7.1 If transport of collected samples to the laboratory testing area is required, then use the swab contained in the swab tubes, culturette tubes, or similar system (swab in a test tube with a transport medium), in place of the dry swab as described in 4.7. Any transport medium transferred to the agar or broth should not adversely affect the results.

7.2 Test swabs in tubes without media as soon as possible to avoid drying of swab and possibly killing any contaminating microorganisms. Test swabs in tubes with media within the time specified by the manufacturer (generally 48 to 72 h).

8. Testing Procedure for Liquid Samples or Swabs, or Both

8.1 Grasping the opposite end, dip the cotton end of a dry sterile swab into the liquid (or mixture from Procedure 9), remove the cover from a sterile tryptic soy agar (TSA) plate, and streak the agar surface with the wet swab. Make sure that this is done so that the streaks are in a set pattern (for example, three streaks from left to right with 12.7-mm, (½-in.) spacing, criss crossed by three streaks from top to bottom, also with 12.7-mm (½-in.) spacing). Replace the cover. Do this as quickly as possible to avoid introducing airborne contamination to the plates.

NOTE 5—Optimally, these procedures should be carried out in a laminar flow hood or other sterile environment. Minimally, a relatively clean area as specified in 4.10 must be used. The use of antiseptic solution (see 4.11) to regularly sanitize countertops and other work surfaces is recommended. Unfiltered air, hands, unsanitized surfaces and equipment may introduce contamination during the transfer and give a false indication of contamination. The use of aseptic technique during transfer is very important in ensuring the reliability of these tests (see also 10.5 and the appendix to detect anaerobic bacteria).

8.2 Dip the swab again into the mixture and repeat the streaking as in 8.1 using an acidified potato dextrose agar (PDA) plate or malt agar plate.

8.3 Turn the streaked TSA plates upside down, and the PDA or malt agar plates right side up. Place all streaked plates in an incubator, and incubate at 30°C for the specified time. Make sure that the incubation time for fungi (PDA or malt agar plates) is 3 to 7 days, and for bacteria (TSA plates), 24 to 48 h.

NOTE 6—The 30°C temperature is generally appropriate for detecting environmental contaminants. If two incubators are available, use 28°C for the fungi and 32°C for the bacteria. If humidity control is available, use 95 % relative humidity (rh) for the fungi and 50 % rh for the bacteria.

NOTE 7—To achieve some degree of humidity control in a non-humidity controlled incubator or oven, place a container (such as a borosilicate baking dish) filled with distilled water at the bottom of the incubator. This helps to prevent the drying out of the plates (which could inhibit the growth of any microorganisms and give a false indication of sterility). Change this water regularly to avoid growth of bacteria, etc. (or a piece of copper wool can be used to help control microorganism growth).

9. Testing Procedure for Dry Materials

9.1 Obtain or weigh out a suitable amount of dry material (0.1 to 0.5 g) using sterilized equipment (either a sterile spatula or sterile wooden tongue depressor) and add this to a tube of sterile diluent (see 4.9). Recap the tube and shake vigorously.

NOTE 8—If the material does not go into solution, shake or swirl the tube so that a uniform mixture is obtained just prior to the streaking procedure (8.1) (see also 5.1, Note 2, and Note 5).

9.2 Using the resulting liquid, continue as listed in 8.1 for liquid materials.

9.3 For each additional dry sample use a new sterile spatula or tongue depressor.

10. Evaluation of Results

10.1 Bacterial contamination (aerobic) is generally characterized by milky spots of varying size (bacterial colonies) on the agar surface. These are usually slimy or shiny in appearance.

10.2 Fungal contamination is generally characterized by spots that are usually filamentous and more fuzzy in appearance, with the exception of yeasts which normally look similar to the bacterial colonies.

NOTE 9—If present, bacteria should grow on the TSA plates, but bacteria can also grow on the PDA or malt extract plates, particularly if they are not acidified. Fungi can also grow on the TSA plates, and yeast in particular can look like a bacterial contamination. Differentiation between bacterial and fungal growth can require more sophisticated techniques than are covered in this test method. Assistance can be obtained from your biocide supplier.

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