Thermal Spore Exposure Vessels

Thermal masses are minimized to enable rapid heating and cooling.

NASA's Jet Propulsion Laboratory, Pasadena, California

Thermal spore exposure vessels (TSEVs) are laboratory containers designed for use in measuring rates of death or survival of microbial spores at elevated temperatures. A major consideration in the design of a TSEV is minimizing thermal mass in order to minimize heating and cooling times. This is necessary in order to minimize the number of microbes killed before and after exposure at the test temperature, so that the results of the test accurately reflect the effect of the test temperature.

A typical prototype TSEV (see figure) includes a flat-bottomed stainless-steel cylinder 4 in. (10.16 cm) long, 0.5 in. (1.27 cm) in diameter, having a wall thickness of 0.010±0.002 in. (0.254±0.051 mm). Microbial spores are deposited in the bottom of the cylinder, then the top of the cylinder is closed with a sterile rubber stopper. Hypodermic needles are used to puncture the rubber stopper to evacuate the inside of the cylinder or to purge the inside of the cylinder with a gas. In a typical application, the inside of the cylinder is purged with dry nitrogen prior to a test.

During a test, the lower portion of the cylinder is immersed in a silicone-oil bath that has been preheated to and maintained at the test temperature. Test temperatures up to 220 °C have been used. Because the spores are in direct contact with the thin cylinder wall, they quickly become heated to the test temperature.

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A **TSEV** is a test-tube-like stainless-steel vessel that can be purged with a gas and immersed in a hot oil bath.

Enumerating Spore-Forming Bacteria Airborne With Particles

Complementary data are obtained by use of two instruments and correlated.

NASA's Jet Propulsion Laboratory, Pasadena, California

A laboratory method has been conceived to enable the enumeration of

- Cultivable bacteria and bacterial spores that are, variously, airborne by themselves or carried by, parts of, or otherwise associated with, other airborne particles; and
- Spore-forming bacteria among all of the aforementioned cultivable microbes.

The method was originally intended to be used along with other methods as means of quantitative assessment of the potential for contamination of remote planets by bacterial spores carried aboard spacecraft from Earth. There may also be potential terrestrial applications in assessing actual or potential airborne biological contamination on Earth. Of particular interest, for a given sample, the method provides information on the average number of spores per particle as a function of particle size. This information is important for studies of airborne biological contamination because (1) spores are particles and are believed to be associated with other particles and (2) entrainment and transport of particles in air are strongly affected by particle size.

The method involves the use of two commercially available instruments:

- One instrument is a quartz-crystal-microbalance (QCM) ten-stage cascade impactor, which can quantify airborne particles of different sizes but cannot measure bacterial content.
- The other instrument is a particle counter that includes six stages, in which are placed tryptic soy agar (TSA) plates to collect particles in six different size ranges. TSA is a basic medium used for culturing many kinds of microorganisms. This instrument indicates the numbers of microbial colonies that grow on the plates after incubation, which numbers correlate with the numbers of the cultivable total microbes collected in air. However, this instrument does not count all of the particles (including particles other than bacteria and spores) collected on each agar plate.

The method prescribes two tasks, the first being to determine the total distribution of cultivable microbes (both spore-forming and non-spore-forming) on airborne particles of different sizes. In this first task, the two instruments are positioned side by side and run simultaneously. The six-stage counter gives the total number of cultivable microbes per unit area for each particle size range. The QCM ten-stage cascade impactor gives the total numbers of particles (including particles other than cultivable microbes) per unit area per size range. The size ranges of the two instruments are different. The data acquired by the two instruments are consolidated in a procedure that includes taking account of the differences between the size ranges. This procedure yields information on the average

number of cultivable microbes per particle as a function of particle size.

The second task is to determine the distribution of spore-forming bacteria (and not of non-spore-forming bacteria) on airborne particles of various sizes. This task incorporates the procedures of the first task and includes additional procedures. Because the colonies collected by the six-stage particle counter contain non-spore-forming as well as spore-forming bacteria, this task includes a procedure for differentiating between the spore-forming and non-spore-forming colonies.

To start the differentiation procedure, a sample is taken from every colony on the agar plates and incubated in a nutrient sporulation medium (NSM) for seven days. An NSM is a low-nutrient medium, and only spore-forming species (which are hardier than non-spore-forming species) are capable of forming spores that can survive the week-long low-nutrient condition. Next, the NSM-incubated samples are subjected to a heat shock consisting of a temperature of 80 °C for 15 minutes. Spores can survive the heat shock, but non-spore-forming bacteria and spore-forming bacteria that are not in spore form cannot survive. Therefore, any colonies that resume growth after the incubation in the NSM and the heat shock are deemed to be colonies of spore-forming bacteria. The resulting knowledge of which of the colonies on the agar plates consist of spore-forming bacteria is used to form the counts in the six size ranges, then the data from the two instruments are consolidated, in the same manner as in the first task, to obtain the average number of spore-forming bacteria per particle of each size range.

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