mally insulated with closed-cell foam to reduce heat losses.

To enable a sufficiently high degree of sterilization and inactivation of endotoxins during the time of transit of water along the tube, it is necessary to heat the water to a temperature significantly above traditional autoclave temperatures. In order to do this, it is necessary to choose an appropriate combination of microwave power and flow rate, and to maintain the flowing water at a pressure high enough to prevent boiling at the desired maximum temperature. For example, typical steady-state operating conditions in experiments on this system included a flow rate of 13 mL/min, a pressure of 0.69 MPa, and a microwave power of 150 W, resulting in a sterilization temperature between 155 and 158 °C. Under these conditions, the system demonstrated effective sterilization and inactivation of endotoxins when challenged with influent water containing a mixed culture of *Bacillus stearothermophilus*, *E. coli*, and *Pseudomonas aeruginosa*.

Downstream of the sterilization chamber, the water is cooled at reduced pressure in a sterilizable connector section that contains a cooling coil situated between two pressure regulators. At the beginning of operation, for the purpose of sterilization, the sterilizable connector section is surrounded by removable insulation and maintained at full sterilization temperature and pressure using pressure regulator 2. For subsequent steady-state operation, insulation is removed from this section, causing the temperature to decline to the point where pressure regulator 2 can be opened to the atmosphere, requiring adjustment of pressure regulator 1 to maintain full sterilization pressure in the sterilization chamber.

This work was done by James R. Akse, Roger W. Dahl, and Richard R. Wheeler, Jr. of UMPQUA Research Co. for Glenn Research Center. Further information is contained in a TSP (see page 1).

Inquiries concerning rights for the commercial use of this invention should be addressed to NASA Glenn Research Center, Innovative Partnerships Office, Attn: Steve Fedor, Mail Stop 4–8, 21000 Brookpark Road, Cleveland, Ohio 44135. Refer to LEW-18159-1.

Discrimination of Spore-Forming Bacilli Using *spo*IVA

Sporulation-specific primers are mixed into a PCR cocktail.

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A method of discriminating between spore-forming and non-spore-forming bacteria is based on a combination of simultaneous sporulation-specific and non-sporulation-specific quantitative polymerase chain reactions (Q-PCRs). The method was invented partly in response to the observation that for the purposes of preventing or reducing biological contamination affecting many human endeavors, ultimately, only the spore-forming portions of bacterial populations are the ones that are problematic (or, at least, more problematic than are the non-spore-forming portions).

In some environments, spore-forming bacteria constitute small fractions of the total bacterial populations. The use of sporulation-specific primers in Q-PCR affords the ability to assess the spore-forming fraction of a bacterial population present in an environment of interest. This assessment can provide a more thorough and accurate understanding of the bacterial contamination in the environment, thereby making it possible to focus contamination-testing, contamination-prevention, sterilization, and decontamination resources more economically and efficiently.

The method includes the use of sporulation-specific primers in the form of designed, optimized deoxyribonucleic acid (DNA) oligonucleotides specific for the bacterial *spo*IVA gene (see table). [In "*spo*IVA," "IV" signifies Roman numeral four and the entire quoted name refers to gene A for the fourth stage of sporulation.] These primers are mixed into a PCR cocktail with a given sample of bacterial cells. A control PCR cocktail into which are mixed universal 16S rRNA primers is also prepared. ["16S rRNA" denotes a ri-

Primer and Probe Sequences for Discriminating and Quantifying Spore-Forming Bacteria

Primer Name	Position	Sequence
SpoIVa382f	Forward Primer Sequence	5'-TGA AGA GCC NAT YCC RTT-3'
SpoIVa752r	Reverse Primer Sequence	5'-ACC ATT ACC CAG CTY GG-3'

Primer and Probe Sequences for Discriminating and Quantifying *B. anthracis* Bacteria

Primer/Probe Name	Position	Sequence
SpoIVa358f	Forward Primer Sequence	5'-ACB CCW TGG TAY GAA GAR CC-3'
SpoIVa508r	Reverse Primer Sequence	5'-TTC TGC CTC TAT ATA ATC TCT TCT TGG-3'
SpoIVa385f	Fluorescent Probe Sequence	5'-TTC CAT GAA GCT GCA GAA ATC GG-Fluor-3'

Note: A = adenosine; C = cytosine; T = tyrosine; G = guanine; N = the use of any of the 4 nucleotides, A, T, G, or C; R = the use of either purine nucleotide, A or G; W = the use of A or T; Y = the use of either pyrimidine nucleotide, C or T.

These Primers can be used, variously, for discriminating spore-forming bacteria in general or *Bacillus anthracis* in particular.

bosomal ribonucleic acid (rRNA) sequence that is common to all organisms.] Following several cycles of heating and cooling according to the PCR protocol to amplify amounts of DNA molecules, the amplification products can be analyzed to determine the types of bacterial cells present within the samples.

If the amplification product is strong, relative to the product of a control PCR sequence, then it is concluded that the bacterial population in the sample consists predominantly of spore-forming cells. If the amplification product is weak or nonexistent, then it is concluded that the bacterial population in the sample consists predominantly or entirely of non-spore-forming cells.

This work was done by Kasthuri Venkateswaran, Myron La Duc, and Tara Stuecker of Caltech for NASA's Jet Propulsion Laboratory. Further information is contained in a TSP (see page 1).

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