

# Spore News

Volume 2, Number 4  
December 2005



## **Spores Don't Lie... and Probes Don't Always Know (or tell) the Truth**

Every day, sterilization cycles are run. They may contain glassware and equipment to stock our laboratories, tubes of culture media, or vials of sterile injectable pharmaceuticals. Regardless of the items being processed and regardless of whether they are processed by steam, ethylene oxide (EtO) gas, dry heat or any other lethal agent, the purpose of the sterilization cycle is to kill microorganisms that exist in or on the product.

Each of these sterilization processes has critical variables that must be controlled. For steam sterilization, one must control saturated steam temperature, pressure, residual air and dwell time. In ethylene oxide systems, relative humidity and concentration of EtO gas in addition to temperature and dwell time must be controlled. In each case, one controls the sterilization cycle by physical means based upon the response of calibrated devices. To ensure cycle performance, the probes are calibrated at regular intervals using reference instrumentation that is traceable to a national standard.

With all of these controls in place, we expect to load our sterilizer, close the chamber door, select the appropriate cycle and await our finished, sterilized product. Having reviewed the cycle data as reported by the calibrated probes and determining that all parameters were within acceptable limits, retrieval and incubation of the biological indicators sometimes seems secondary in nature. Secondary that is, until one opens the door to the incubator and observes a positive test result.

“No spore strip should be able to survive a 212-minute, VALIDATED ethylene oxide cycle” is the disgruntled claim that I hear through the phone handset. This is a true statement. Our Spore Laboratory would have to produce a spore crop with a 35-minute EtO D-value for a biological indicator with a  $10^6$  spore population to survive such an insult (as compared to the 2.5 to 5.8-minute range specified in USP).

The answer to the clients' dilemma is... “Spores don't lie and probes don't always know (or tell) the truth.” In each instance it seems as though metrology just finished calibrating all the probes and annual re-validation was just recently completed and all the process variables were within acceptable limits. Regardless, the spores are currently growing in the incubator.

The probes that control and monitor our sterilization cycle performance are monitoring the conditions in one specific location. What one must not do is *assume* that if acceptable readings are generated by the probes, that these conditions exist at all locations within the load. In the above EtO example, it is very likely that during the 212-minute dwell time, all of the probes indicated acceptable relative humidity (RH), concentration of EtO gas, and exposure temperature. Thus, the sterilizing conditions in the chamber would appear to be acceptable. Because of their size and attached wiring, probes are routinely placed in the chamber space. However, what lethal conditions are prevailing deep within the load? Has a sufficient amount of moisture reached the area where the biological indicator is positioned? What about EtO gas molecules? Penetration to the site may have been impeded. And let us not ignore temperature penetration. As temperature increases, the percent RH will decrease if no additional moisture is introduced to that specific location. It is very possible that 60% RH did exist at the location of the BI but as the temperature of that site continued to increase to the acceptable exposure condition, the %RH may decrease to lower than acceptable limits. We can't assume moisture molecules and EtO gas molecules diffuse at the same rates and that these events happen simultaneously with heat transfer.

In a steam system, the temperature probes will respond only to temperature and the monitoring equipment will begin to report the accumulated lethality. As the cycle progresses, one sees that the Fo values being reported by the various probes continue to accumulate and eventually surpass the minimum lethality requirement established during cycle development and validation. How then did the BI survive 30 Fo; more than twice the lethality typically required to kill the spore challenge? Again, the answer to the question is... "Spores don't lie and probes don't always know (or tell) the truth." The probe is measuring temperature and cannot determine if that temperature reading is due to the presence of wet steam, saturated steam, superheated steam or even dry air. Meanwhile, the pressure transducer is indicating a pressure reading that does correspond to saturated steam conditions, but don't forget that this reading is also monitoring a discreet location. This device will accurately measure total pressure but it is unable to evaluate what molecules are responsible for the reading. Again, we can not assume that homogenous conditions exist at all locations throughout the load and that those conditions are exactly as what the probes are indicating.

The probes in your sterilization equipment are state of the art, accurately calibrated, highly sensitive instruments and without them, controlling ones cycle would not be possible. But we must not forget that each one measures a limited environment and is only capable of sensing and measuring one parameter in a cycle. Multiple parameters are critical for the correct attainment of lethal conditions. The "truth" in lethality depends upon proper integration of all of the various components and critical process parameters at each location within the load. There is one "instrument" capable of sensing, integrating, and responding to *all* of the prevailing conditions at *all* times during the cycle. This inexpensive, disposable instrument is wireless and in most cases small in size such that it can be easily positioned throughout the load and located in areas where sterilization is least likely to occur. It is supplied by the manufacturer already calibrated

with a Certificate of Analysis. This amazing “instrument” that can accurately monitor delivery of lethality at various locations throughout the load regardless of how dynamic conditions are is the Biological Indicator spore.

If you want to know the “truth” about your sterilization cycle performance, you’ll need to ask a spore. When it comes to cycle lethality, spores know “the whole truth and nothing but the truth.” A probe only knows *one* part of the truth and true cycle performance and delivery of lethality is never dependent upon only *one* critical variable.

**Please email us with topics you would like to see addressed in “Spore News”.**

