Spore News™

M E S A L A B S

Volume 8, No. 2

The Problem with EP 7.0, 5.1.2

by Garrett Krushefski

This Spore News will outline the biological indicator (BI) performance requirements that appear in United States Pharmacopeia (USP) 34, ANSI/AAMI/ISO 11138:2006 and European Pharmacopeia (EP) 7.0. After comparing the information presented in these various documents we will discuss why some of these requirements that appear in EP 7.0, 5.1.2 are likely to cause problems for the BI community.

Our BIs are manufactured to comply with USP and ANSI/AAMI/ISO standards. Where as the minimum population and minimum D-value requirements of EP are not a problem, it is the additional statements about performance requirements that severely limit the BI manufacturer to make and the BI user to find a suitable BI. We thus caution all BI users that if you intend to try and comply with EP 7.0, 5.1.2, you may find it quite difficult to source acceptable BIs regardless of which BI manufacturer you prefer.

Table 1 shows the various requirements for BI spore population per unit

	Steam	EtO gas	Dry Heat	Radiation
USP :	not less than 10^4 not more than 10^9	not less than 10^4 not more than 10^9	not less than 10^4 not more than 10^9	n/a
ISO:	$\geq 1.0 \times 10^5$	$\geq 1.0 \times 10^6$	$\geq 1.0 \times 10^6$	n/a
EP:	$> 5 \times 10^5$	$> 1 \times 10^6$	$> 1 \times 10^6$	$> 1 \times 10^7$

Table 2 shows the various requirements for BI D-value

	Steam (121°C) (minutes)	EtO gas BIs (* 54 °C / † 30 °C) (minutes)	Dry Heat (160°C) (minutes)	Radiation BIs (kGy)
USP :	1.5 to 3.0	2.5 to 5.8*	1.0 to 3.0	n/a
ISO:	≥ 1.5	not less than 2.5* not less than 12.5†	not less than 2.5	n/a
EP:	not less than 1.5	not less than 2.5*	not less than 2.5	not less than 1.9

*when tested at 600 mg/L EtO, 54°C, 60% relative humidity
†when tested at 600 mg/L EtO, 30°C, 60% relative humidity
NOTE: The D-value ranges for USP do not appear in the official monographs. Rather, these values appear in chapter <1035> and thus, are considered informative only, not mandatory.

In addition to the population and D-value requirements outlined above, the standards contain the following citations. Those items in red font will be the focus of subsequent discussion.

STEAM

EP:

1) It is verified that exposing the BIs to steam at 121 +/- 1°C for 6 minutes leaves revivable spores.
2) It is verified that there is no growth of the reference micro-organisms after the biological indicators have been exposed to steam at 121 +/- 1°C for 15 minutes.

ISO:

1) The z-value shall be determined at not less than three temperatures, in the range of 110°C to 130°C. These data shall be used to calculate the z-value, which shall be ≥ 6°C.

EtO GAS

EP:

1) It is verified that there is no growth of the reference micro-organisms after the biological indicators have been exposed for 25 minutes (600 mg/L EtO, 54°C, 60% relative humidity)
2) It is verified that exposing the indicators for 50 minutes leaves revivable spores (600 mg/L EtO, 30°C, 60% relative humidity).

ISO:

1) D-value stated to one decimal place at 54°C or 30°C, or at both temperatures.

DRY HEAT

ISO:

1) The z-value shall be determined at not less than three temperatures, in the range of 150°C to 180°C. These data shall be used to calculate the z-value, which shall be ≥ 20°C.

RADIATION

EP:

1) It is verified that there is no growth of the reference micro-organisms after the biological indicators have been exposed to 25 kGy (minimum absorbed dose).

Let us now discuss the above items that appeared in red font. The first citation appears in EP 7.0, 5.1.2 with respect to saturated steam biological indicators.
"It is verified that there is no growth of the reference micro-organisms after the biological indicators have been exposed to steam at 121 +/- 1°C for 15 minutes."

For a BI to show no growth, one must deliver 2 SLRs (spore log reductions) beyond the log value of the spore population. Where as ISO and USP call for a minimum population of 1.0×10^5, EP requires a minimum population of 5.0×10^5. Thus, the $\log_{10} 5.0 \times 10^5$ is 5.70. If we deliver only 5.7 SLRs to this theoretical BI, we will have (on average) 1 surviving spore per BI and this equates to a 63% survival rate. One additional SLR (i.e. 6.7 SLRs) to the 5.0×10^5 spore population leaves a 10% BI survival rate. It is not until 7.7 SLRs (i.e. 2-SLRs beyond the log of the BI population) that we reach a 1% BI survival rate or a level where we might consistently expect to see a no growth result. As stated in EP, this no growth result must be achieved with only 15 minutes exposure at 121°C. If we divide the 15 minute exposure period by the 7.7 SLRs needed to achieve

no-growth, we calculate that the D-value of the BI in question must not exceed 1.95 minutes (i.e. 15 ÷ 7.7 SLRs = 1.95 minute D-value). This requirement essentially places an upper limit on D-value. Thus, we can see that ANY steam BI with population greater than 5.0×10^5 and/or D-value greater than 1.9 minutes will likely still show a growth positive result when exposed to 15 minutes at 121°C. Thus, a customer who wants to purchase a steam BI that complies to EP 7.0, 5.1.2 will need to search for a 1.5 to 1.9 minute D-value label claim.
If the BI in question has a spore population of 2.0×10^6, the D-value could not exceed 1.81 minutes.
EXAMPLE:
$\log_{10} 2.0 \times 10^6 = 6.30$
15 minute steam exposure ÷ (6.30 + 2 SLRs) = 1.81 minute D-value

The next problematic EP citation is essentially the same but in reference to EtO BIs.
"It is verified that there is no growth of the reference micro-organisms after the biological indicators have been exposed for 25 minutes (600 mg/L EtO, 54°C, 60% relative humidity)." For this example we'll consider an EtO BI having spore population of 1.0×10^6. As discussed above, one needs to achieve 2 SLRs beyond the log value of the BI population to reach a 1% survivor level. Thus:
$\log_{10} 1.0 \times 10^6 = 6.0$
25 minute exposure ÷ (6.0 + 2 SLRs) = 3.13 minute D-value

As the BI is a monitor of sterilization cycle performance, we wonder why make any such statement that places an upper limit on BI resistance capabilities? One could easily understand why a minimum population and minimum D-value would be specified; essentially to make certain that the BI challenge is not too weak as it would not be a formidable challenge to the sterilization process. But why impose an upper limit to BI resistance…especially one that substantially limits the acceptable range of resistance?

Finally, let us consider the third red font statement; "It is verified that exposing the indicators for 50 minutes leaves revivable spores (600 mg/L EtO, 30°C, 60% relative humidity)." The intent of this statement is to show that the BI is capable of detecting a diminished lethality such that would exist by a 30°C exposure condition as opposed to the 54°C condition at which the BIs were tested. (NOTE: a previous version of EP stated that EtO BIs should be tested in a reduced humidity cycle to verify that survivors are attained. That requirement does not appear in EP 7.0 and was apparently replaced with this reduced temperature test.)

Spores are the "gold standard" when it comes to monitoring sterilization conditions. If we make a BI that meets the minimum population and minimum D-value specification at a particular set of exposure conditions, then why the need to test the same BI at other conditions such as this reduced temperature cycle? The spores sense and respond to the conditions of the sterilization cycle and integrate the total lethal impact. The prevailing temperature, relative humidity, and/or concentration of EtO gas can fluctuate and at the end of the cycle, the spores will either have survived or have been killed. Since we started with a BI that met or exceeded the minimum population and D-value requirements, there is no need to start testing all sorts of other potential scenarios of reduced temperature and/or reduced humidity and/or reduced EtO concentration. Regardless of any fluctuation of any of these critical process parameters if the overall insult was capable of killing the spores, then it was capable of sterilizing the goods in the sterilizer.

So what if the temperature is only 30°C and the spores still get killed in a 50 minute cycle...what does this mean? It means that despite the erroneous temperature the overall combination of conditions were still sufficient to kill the spore challenge. We know from the BIER testing that the BI in question met the minimum population and minimum D-value criteria and if the spores in that BI were still killed despite the errant condition then where is the problem? *Were the spores killed because the BI is too weak?* No, we've already established that the spores are not too weak because they meet or exceeded the minimum EP requirement.

As stated above, our BIs are manufactured to comply with USP and ANSI/AAMI/ISO standards. BI users that

intend to try and comply with EP 7.0, 5.1.2, may find it quite difficult to source acceptable BIs regardless of which BI manufacturer you prefer.

About the author:

Garrett Krushefski is the Manager of International Sales for Mesa Labs. In his twelve years at the Bozeman, Montana location, Mr. Krushefski began his work in the biological indicator production laboratory, later became the Supervisor and eventually the Laboratory Manager. Most recently he provided technical assistance and guidance to customers with respect to sterilization and the proper application of biological indicators as the Scientific & Technical Services Manager.

Mr. Krushefski is a committee member and active participant with the Association for the Advancement of Medical Instrumentation (AAMI) in both the Biological Indicator and Resistometer working groups.

Mr. Krushefski holds a B.A. in Biology from The University of Texas at Austin.

Contact the author:

Garrett Krushefski
International Channel Sales Manager
10 Evergreen Drive
Bozeman, MT 59715
Phone: 406-585-9535
Fax: 406-585-9219
Email: gk@mesalabs.com