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Comparison of European and US biological indicators for ethylene oxide sterilization

Christopher A. Demitrius[1], Anthony P. Duran[1], Virginia C. Chamberlain[2] and Victoria M. Hitchins[2]

[1]*US Food and Drug Administration, Midwest Laboratory for Microbiological Investigations, Minneapolis, MN 55401 and* [2]*US Food and Drug Administration, Center for Devices and Radiological Health, Rockville, MD 20857, USA*

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SUMMARY

Biological indicators (BIs) are used to monitor ethylene oxide (EO) gas sterilization processes for medical devices. Several European and United States BIs for EO sterilization were evaluated for resistance according to both United States Pharmacopeia (USP) XXI and United Kingdom's (UK) tests for D-values. US BIs are *B. subtilis* var. niger spores on paper strips or disc carriers, while European BIs use aluminum strips, quartz sand, or cotton yarn. Numerous BIs per run and runs per lot, as well as 2–3 different lots of BIs from each manufacturer, were examined. Both British and US BIs met their respective label claims for rates of inactivation when tested against British and USP EO test parameters, respectively. However, Danish BIs, on cotton yarn or quartz sand, were not inactivated following USP specifications during the exposure dwell times tested (600 mg L^{-1} EO, 54 °C, 60% RH, 0–110 min). The Danish BIs will require further testing in order for us to determine if their *B. subtilis* spores are unusually resistant to EO or if the spore carrier substrates protect the spores from the sterilizing gas. In conclusion, the British and American BIs for EO sterilization are equivalent in resistance despite differences in carrier substrate, recovery conditions, calculation methods for D-values, and the labeled sterilization conditions for use.

INTRODUCTION

Nosocomial infections caused by inadequately sterilized medical devices are a health problem for patients. Microbial infections can be spread to a patient via an inadequately sterilized new device or between patients from an inadequately sterilized reused device. Possible consequences of inadequately sterilized medical devices are: an implanted infected device (e.g. prosthetic heart valve) that must be replaced; infection with diseases such as hepatitis, acquired immune deficiency syndrome, or tuberculosis; or death from the infection or complications of the disease itself (e.g. septicemia).

Biological indicators (BIs) are used to monitor ethylene oxide (EO) gas sterilization processes for medical devices. US BIs are *B. subtilis* spores on paper carriers. European BIs are *B. subtilis* spores on aluminum strips, quartz sand or cotton yarn as carriers, an attempt to simulate the most difficult site to sterilize on medical devices. Some claim that European BIs, because they are on carriers that may more closely resemble medical device surfaces, may improve safety from transmission of pathogenic microorganisms on or in medical devices by offering greater resistance to ethylene oxide sterilization. In addition to differences in carrier substrates, there are differences in the sterilization parameters of gas concentration and composition, temperature, humidity, pressure, recovery conditions and calculation methods for decimal reduction value (D-value) between European and US BIs for EO sterilization.

Because of differences in the sterilization test parameters, outgrowth conditions, calculations of D-values, and the materials used as carriers of the *B. subtilis* spores, the purpose of this study was to analyze and compare US, British and Danish BIs for ethylene oxide sterilization by subjecting them to British, Danish and US test parameters for inactivating spores. We analyzed BIs in order to determine if: (1) they meet manufacturer's label specifications for inactivation, (2) US BIs would perform adequately under European test specifications, and (3) European BIs would perform adequately under USP test parameters.

There has been recent interest in establishing international standards for BIs for ethylene oxide gas sterilization. Thus, it is important to analyze and compare US BIs and European BIs using European and US parameters for inactivating spores. The information from this study will aid in defining appropriate parameters for an international standard that will cover making BIs for EO gas sterilization, inactivating them, recovering non-inactivated spores, and calculating D-values.

Correspondence to: V. Hitchins, Center for Devices and Radiological Health (HFZ-112), US Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, USA.

MATERIALS AND METHODS

Biological indicators

The following BIs were purchased: American Sterilizer Company (AMSCO) Erie, PA, USA: *B. subtilis* var. niger, ATCC 9372, on paper strip; North American Science Associates, Inc. (NAmSA), Northwood, OH, USA): *B. subtilis* var. niger, ATCC 9372, on paper strip; Raven Bio-Lab, Inc. (Raven), Omaha, NE, USA: *B. subtilis* var. niger, ATCC 9372, on paper strip; Albert Browne Ltd. (Browne), Leicester, UK: *B. subtilis* var. niger NCTC 10073, on aluminum strip; and Steriseal, Worcestershire, UK: *B. subtilis* var. niger NCTC 10073, on aluminum strip. Two different types of currently available BIs from the Statens Seruminstitut, Copenhagen, Denmark were generously provided: *B. subtilis* var. niger SSI K2, on cotton yarn and *B. subtilis* var. niger SSI MK1, on quartz sand. Three different lots of BIs were obtained from each manufacturer except from Browne which had only two different lots. All BIs were labeled to contain 10^6 colony-forming units of *B. subtilis* spores and this was confirmed.

TABLE 1

Number of biological indicators and runs of US and European biological indicators tested using typical US and British sterilization parameters for ethylene oxide gas sterilization processes

Manufacturer name	Lot no.	UK test BIs/run	UK test runs/lot	US test BIs/run	US test runs/lot
AMSCO (US)	A	9	6	10	9
	B	9	6	10	11
	C	9	6	10	9
NAmSA (US)	A	9	6	10	6
	B	9	6	10	4
	C	9	6	10	5
Raven (US)	A	9	6	20	7
	B	9	6	10	7
	C	9	6	10	10
Browne (UK)	A	9	6	10	10
				5	7
	B	9	6	10	11
				5	5
Steriseal (UK)	A	9	6	10	15
	B	9	6	10	7
	C	9	6	10	10
Seruminstitut (Denmark)	cotton				
	A	N/A	N/A	10	10
	B	N/A	N/A	10	9
	C	N/A	N/A	10	7
	sand				
	A	N/A	N/A	10	19
	B	N/A	N/A	10	18
	C	N/A	N/A	10	17

Ethylene oxide test vessel and ethylene oxide gas

The test vessel was a BI Evaluator Resistometer vessel (BIER-vessel/EO; Joslyn Valve Corp., Macedon, NY) that was manufactured for the commercial sterilant EO/dichlorodifluoromethane (12/88 mixture by weight). The vessel was modified for the temperatures of 29 ± 0.5 °C, 33 ± 0.5 °C, and 37 ± 0.5 °C.

Ethylene oxide sterilization conditions

The United States Pharmacopeia (USP) XXI parameters for D-value determinations for biological indicators for ethylene oxide sterilization, paper strip [7] are: 600 mg L^{-1} EO, 54 °C, and 60% relative humidity. The United Kingdom's Department of Health and Social Security (DHSS) specification for biological monitors for the control of ethylene oxide sterilisation specifies: 475 mg L^{-1} EO, 29 ± 0.5 °C, ambient humidity, and 9.67 PSIA initial vacuum [4]. For Danish BIs, the parameters for inactivation of BIs are: 450 mg L^{-1} EO, 55 °C, 60% RH, and 1.00 PSIA initial vacuum.

Test procedures

(1) UK DHSS test procedures: Nine BIs were exposed to UK DHSS parameters [4]: 475 mg L^{-1} EO at 29 °C, 33 °C, and at 37 °C for 0, 10, 20, 30, 45, and 60 min. Each exposed spore carrier was then transferred to a separate screw cap tube containing 10 ml sterile distilled water, vortexed for 2 min, and then sonicated for 15 min, with a Bransonic 52 instrument (Branson Cleaning Equipment, Shelton, CT). The resulting spore suspension was diluted and plated on tryptone–glucose agar. Plates were incubated at 35 ± 2 °C for 48 h and resulting colonies were counted with a dark-field Quebec colony counter (AO Instrument Co., Buffalo, NY). D-values were calculated according to the linear regression method using a program of Hewlett-Packard HP-97 Standard Pac (Hewlett-Packard, Corvallis, OR).

(2) US test procedures: BIs were tested according to USP XXI parameters [7]. Ten BIs were exposed to 600 mg L^{-1} EO at 54 °C and 60% relative humidity for dwell times that would yield the results of 'all killed', 'dichotomous' (partial out-growth), and 'all survive', while maintaining an equal time between adjacent exposures [6]. After exposure to EO, each individual spore carrier was transferred to a screw cap tube containing 30 ml of trypticase soy broth to dilute any residual EO. Inoculated tubes were incubated for 7 days at 35 ± 2 °C. Tubes showing growth were counted and D-values were calculated according to the appropriate quantal method, Spearman–Karber or the Stumbo–Murphy–Cochran and Halvorson–Ziegler methods [6].

Media

Several different types of spore outgrowth promotion media for the BIs were specified by the manufacturers of BIs for EO sterilization and used in this study. The following outgrowth media were used: US BIs: trypticase soy agar, broth (TSA and TSB; Difco, Detroit, MI) [7]; British BIs:

TABLE 2

Summary of results of testing biological indicators for ethylene oxide gas sterilization from 3 US, 2 British (UK), and 1 Danish manufacturers following processes according to typical US sterilization parameters: 600 mg L^{-1} EO, 54 °C, 60% RH, and 1.00 PSIA initial vacuum

Manufacturer name	Lot no.	Average D-value (min)	95% C.I. for D-value (min)	Claimed D-value (min)	USP ± 20% range of D-value (min)
AMSCO (US)	A	3.6	3.4–3.8	3.3	2.6–4.0
	B	3.3	3.2–3.5	3.2	2.6–3.8
	C	3.3	3.2–3.4	3.1	2.5–3.7
NAmSA (US)	A	2.8	2.6–3.0	2.9	2.3–3.5
	B	2.8	2.5–3.0	2.9	2.3–3.5
	C	3.0	2.7–3.3	2.9	2.3–3.5
Raven (US)	A	3.1	2.9–3.2	2.8	2.2–3.4
	B	2.6	2.2–3.1	3.5	2.8–4.2
	C	2.5	2.4–2.6	3.5	2.8–4.2
Browne (UK)	A	2.9	2.2–3.4	N/A	N/A
	B	3.2	2.0–3.5	N/A	N/A
Steriseal (UK)	A	1.7	1.6–1.8	N/A	N/A
	B	1.7	1.5–1.8	N/A	N/A
	C	1.7	1.6–1.7	N/A	N/A
Denmark (cotton)	A	>110	N/A	N/A	N/A
	B	>110	N/A	N/A	N/A
	C	>110	N/A	N/A	N/A
					N/A
Denmark (quartz sand)	A	>110	N/A	N/A	N/A
	B	>110	N/A	N/A	N/A
	C	>110	N/A	N/A	N/A

dextrose tryptone agar (1% tryptone, 0.5% glucose, 1.2% agar; Difco) [3]; Danish BIs: tryptone glucose yeast extract agar (0.5% tryptone, 0.3% yeast extract, 0.1% glucose, 1.8% agar; Difco), and tryptone glucose yeast extract broth (0.5% tryptone, 0.3% yeast extract, 0.1% glucose, 0.2% Na_2HPO_4–$2H_2O$, pH 7.8; Difco).

Spore diluents

Two different solutions were used to dilute spores. They were: Butterfield's buffered phosphate [1] for US and Danish BIs, and distilled water for British BIs.

Calculation of D-values

Microbial destruction data were analyzed relative to the survivor curve by the direct enumeration method, or by the fraction negative quantal methods, Stumbo–Murphy–Cochran and Halvorson–Ziegler Most Probable Number Method (SMC HZ MPN), or the Spearman–Karber Mean Time Until Sterility Method (SK) [6].

RESULTS AND DISCUSSION

In our study we compared European and US BIs for ethylene oxide sterilization using both UK and US sterilization parameters for inactivation of the spores, outgrowth, and calculation of D-values. A total of 297 runs using 2896 BIs were used in this study. Table 1 presents the number of BIs subjected to UK's DHSS and to USP XXI test parameters.

The results of our analyses of BIs for ethylene oxide sterilization from three US, one Danish, and two British manufacturers using typical USP XXI parameters are presented in Table 2.

Table 3 presents the data of average D-values of BIs from three US manufacturers and two British manufacturers using typical UK DHSS parameters for inactivation of BIs for ethylene oxide gas sterilization.

The results from this study showed that all BIs from US manufacturers met their labeled D-values for inactivation according to USP XXI parameters. British BIs also met their labeled D-value claims according to DHSS specifications.

Danish BIs were exposed to USP XXI parameters for up to 110 min, without affecting the spore lethality that was achieved on US BIs in a shorter exposure time. Because Danish and British DHSS parameters for inactivating spores are less stringent than USP's in terms of ethylene oxide gas concentration, and, additionally in the case of DHSS, have lower humidity and temperature conditions, Danish BIs

TABLE 3

Summary of results of testing 3 US and 2 British (UK) biological indicators of ethylene oxide gas sterilization processes according to typical UK sterilization parameters, 475 mg L^{-1} EO, 33 °C, ambient humidity, and 9.67 PSIA initial vacuum

Manufacturer name	Lot no.	Average D-value (min)	USP ± 20% range of D-value (min)	Claimed D-value (min)
AMSCO (US)	A	10.2	10.6–15.8	N/A
	B	8.9	10.2–15.4	N/A
	C	9.5	9.9–14.9	N/A
NAmSA (US)	A	10.2	9.3–13.9	N/A
	B	10.2	9.3–13.9	N/A
	C	8.8	9.3–13.9	N/A
Raven (US)	A	9.7	9.0–13.4	N/A
	B	9.9	11.2–16.8	N/A
	C	8.4	11.2–16.8	N/A
Browne (UK)	A	9.2	9–13	11
	B	4.9	7–11	9
Steriseal (UK)	A	8.1	7.0–11.0	9.0
	B	8.3	7.0–11.0	9.0
	C	9.9	7.0–11.0	9.0

were not tested following Danish and DHSS specifications. The greater resistance of Danish BIs to higher ethylene oxide gas concentration raises questions regarding their substantial resistance under USP parameters. Further studies are being undertaken to determine if the Danish BIs' increased resistance to ethylene oxide may be due to the fact that they are prepared in saline and, upon drying, crystals develop that protect *B. subtilis* spores against ethylene oxide gas penetration.

The kinetics of the first order of the rate of chemical reaction of ethylene oxide with reactive sites in spores [5] and temperature, imply that for each 10 °C increase in temperature, there will be a 50% decrease in D-value [2]. In other words, with no change in the gas concentration, a 20 °C increase in the process temperature should give a D-value that is 25% of the initial claimed D-value.

DHSS specification for British BIs is 29 °C or 37 °C. When British BIs were subjected to USP XXI parameters of 54 ± 2 °C, they had D-values that were approximately 25% of their labeled D-values (Table 2). When US BIs were subjected to DHSS specifications of 29 °C and 37 °C, the US BIs had D-values approximately four times their labeled D-values (Table 3). Thus, there appears to be correlation of the first order kinetics of the rate of inactivation of spores dependent on temperature but not on the concentration of ethylene oxide gas.

British and US BIs for EO sterilization were seen to be equivalent in resistance despite differences in carrier substrate, recovery conditions, calculation methods for D-values, and the labeled sterilization conditions for use. This indicates that there are safety assurances built into the BIs and that the parameters for an international standard for BIs for EO gas sterilization using either USP or DHSS parameters will cover BIs from British and US manufacturers.

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