

# Flexible-Chamber Ethylene Oxide Sterilization Systems—Part 2: Demonstration of Homogenous Temperature and EO Concentration Throughout a Flexible Sterilization Bag

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## Abstract

*The use of a flexible chamber as part of an ethylene oxide (EO) sterilization system was first described in 1959. Since the 1960s, flexible-chamber EO sterilization systems have been in continuous use in the United States and around the world. Ethylene oxide flexible-chamber systems offer distinct advantages, including efficient gas usage, very low risk of harm to the sterilization load (no steam injection or deep vacuum), and the ability to process small lots of product efficiently.*

*This paper describes the homogeneity of temperature and EO concentration in flexible-chamber EO sterilization systems and is Part 2 of two recent studies on the characteristics of this sterilization method.*

## Introduction

The ability to predict the lethality of a sterilization process is determined by the ability to control critical process variables. In an ethylene oxide (EO) process, two of the most important sterilization variables are temperature and EO concentration. As the temperature and EO concentration increase, the process becomes more effective for a predetermined load and cycle exposure time. Flexible-chamber EO sterilizers process very small individual load volumes compared to traditional fixed-chamber EO systems.

However, this small chamber size allows for a high degree of control over process variables. Additional background information is provided in Part 1 of this series of studies.

This study was performed to highlight the tight temperature and EO process tolerances achieved within a flexible-chamber sterilization system and how this level of control relates to predictable cycle lethality.

## Materials and Equipment

All temperature and EO distribution studies were performed using one of the following loads:

- A non-absorbing load (orthopedic screws)
- An absorbent load (sutures)
- An empty load (30-L metal frame maximizing volume in an otherwise empty bag)

All studies were performed in an Andersen AN333 EOGas 3 sterilizer using an Andersen AN2011 EOGas cartridge (10.5 g EO). Sterilization loads were placed into both permeable and non-permeable EO flexible sterilization bags containing an AN1071 Humidichip® (an RH stabilization device placed into a holder), and a (an EO process integrator) and were hermetically sealed using an Andersen AN5026 vacuum bag sealer. Gaseous EO samples were removed using Hamilton gas-tight syringes and analyzed

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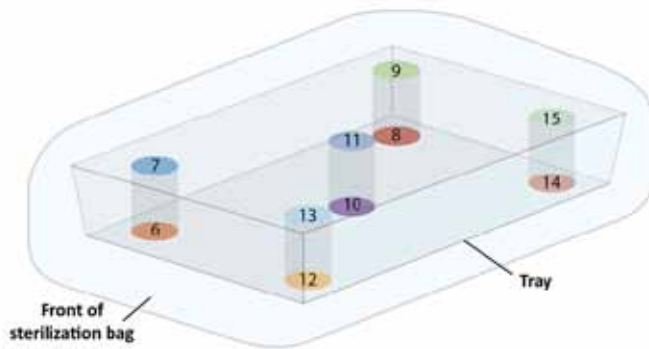


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using a Shimadzu gas chromatograph (GC) and flame ionization detector (FID). Temperature within the flexible sterilization bag and within the sterilizer cabinet was measured with Madgetech intrinsically safe RHTemp1000IS NIST calibrated data loggers.

**Methods**

In order to demonstrate the homogeneity of temperature within the flexible sterilization bag system, 10 data loggers were placed in a geometric pattern throughout the load. Data loggers were placed in the four corners, as well as in the center of the flexible sterilization bag (Figure 1).



**Figure 1.** Data Logger Locations Within the Product Load

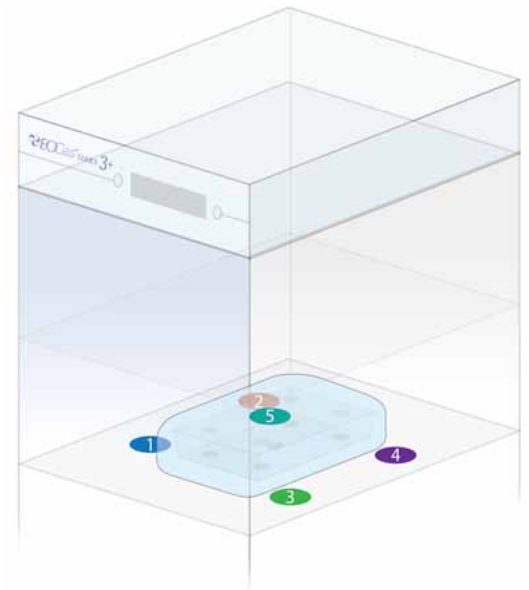
Data loggers were grouped in pairs for each location. Each pair was placed side by side within the load, one right side up and the other upside down (Figure 2). Placing 10 data loggers in this inverted and geometric pattern made it possible to measure the full range of locations in the flexible sterilization bag, including the front, rear, left, right, middle, top, and bottom.



**Figure 2.** A Pair of Data Loggers Measuring High and Low Locations Within the Sterilization Bag

The flexible sterilization bag was hermetically sealed using a vacuum sealer and then placed into the sterilizer cabinet. To demonstrate the effective heating of the sterilization load, five additional data loggers were placed around the flexible sterilization bag in the sterilizer cabinet (Figure 3) so that a comparison between the two could be made. The EOGas cartridge was then activated, and the cycle was

allowed to run for the full 16-hour process time at 50°C.



**Figure 3.** Data Logger Locations Adjacent to the Sterilization Bag

**Demonstration of Homogeneous EO Concentration**

In order to demonstrate that a homogeneous EO concentration exists within the flexible sterilization bag, two EO measurement methods were used: (a) gas chromatography, in which gaseous samples were removed from the bag using gas-tight syringes and quantified in milligrams per liter (mg/L), and (b) EO dosimeters. The , seen in Figure 4, is a chemical integrator designed for use in atmospheric-pressure gas sterilization systems. It responds to time, temperature, and EO gas concentration. A printed triangular calibration mark defines the pass/fail mark. As the dosimeter is exposed to EO, the integrator turns from a yellow-orange color to a dark blue color from the open end (left) toward the closed end (right). The dosimeter was used as an integrator of EO concentration, which is indicated by the distance the blue line travels from left to right in millimeters (mm).

The homogeneity of the EO concentration within the sterilization bag was analyzed using different types of loads and different types of flexible sterilization chambers (EO permeable and EO non-permeable):

- Bag 1 was an impermeable EO steriliza-

tion bag containing a full load of orthopedic screws.

- Bag 2 was a permeable EO sterilization bag containing a full load of sutures.
- Bag 3 was an impermeable sterilization bag without a load—nothing but a 30-L metal frame to maximize air volume in the sterilization bag.

Bags 1 and 2 contained multiple dosimeters placed between the product packages. The dosimeters were placed in a geometric pattern so that all areas of the sterilization load could be monitored (Figure 5). All dosimeters were sandwiched between devices with the sampling end facing upward. Bag 3 was tested empty, so dosimeters were not placed in the sterilization bag.

Each flexible sterilization chamber was vacuum-sealed using an Andersen vacuum sealer, and all cycles ran for approximately 90 minutes at 50°C within the sterilizer cabinet to allow the liquid EO to vaporize into its gaseous form. At that time, 10 one-microliter ( $\mu\text{L}$ ) gaseous EO samples were removed from the designated sample points (Figure 6) and quantified using gas chromatography. To simplify the experimental method, EO samples were removed in groups. During the first hour, samples 1 through 4 (Group 1) were quantified, followed by samples 5 through 8 at the second hour (Group 2), and finally samples 9 and 10 at the third hour (Group 3). For each group location, gaseous samples were removed from the bottom of the sterilization bag (between the packaged devices, near the chevron end of the pouch) and from the top of the sterilization bag just above the packaged devices. This geometric pattern enabled measurement of EO at all locations within the sterilization bag.

## Results

### Homogenous Temperature Distribution

In Figure 7, the temperature data from all data loggers (from within the flexible sterilization bag and from within the sterilizer cabinet) are plotted against time for the full 16-hour exposure phase. Figure 8 represents the first 3 hours of the cycle to clarify the difference in temperature between the sterilization cabinet and the flexible sterilization bag. After the first three hours, all



Figure 4. The Dosimeter (a Chemical Integrator)

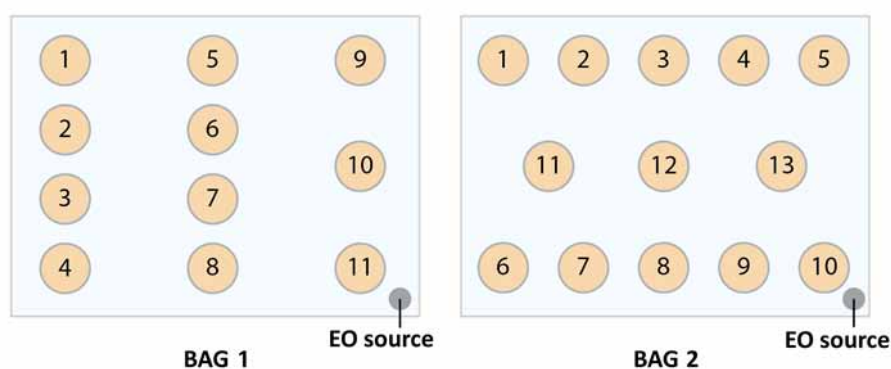


Figure 5. Dosimeter Locations (Bags 1 and 2)

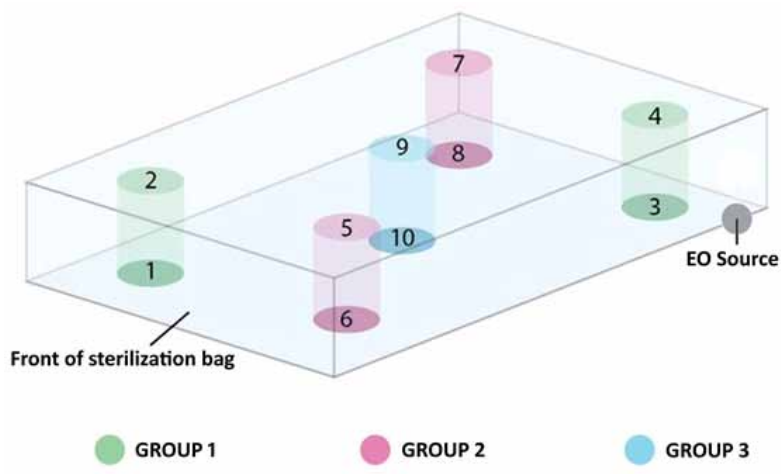


Figure 6. EO Gaseous Sample Locations Within the Sterilization Bag

locations equilibrate with the sterilization cabinet temperature set point of 50°C.

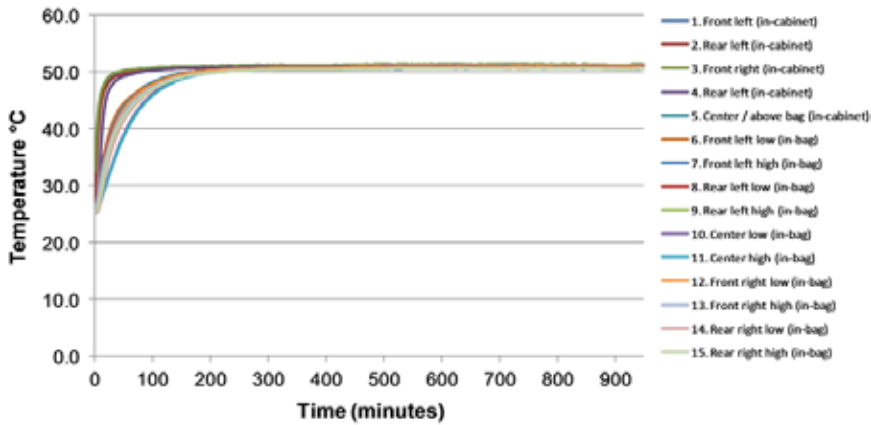


Figure 7. Temperature (°C) at All Locations within the Sterilizer Cabinet and the Flexible Sterilization Bag Over the 16-Hour Exposure Phase

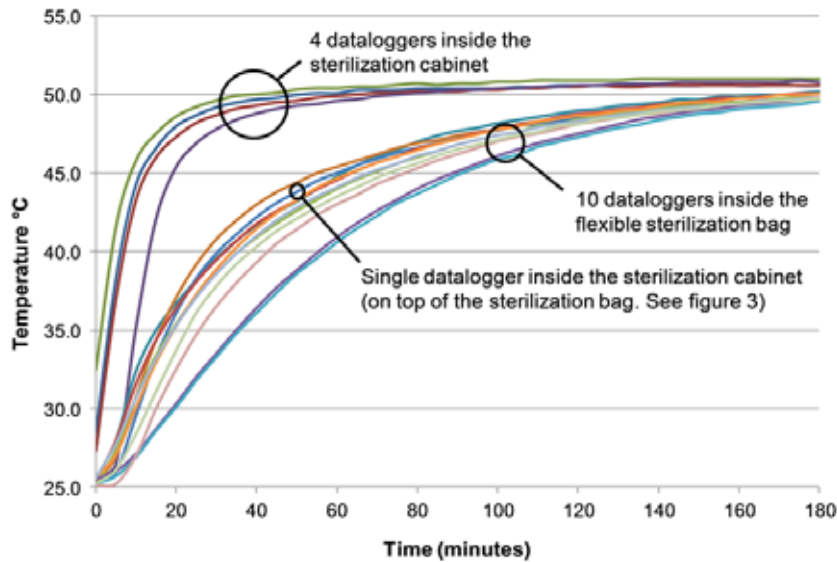


Figure 8. Temperature (°C) at All Locations within the Sterilizer Cabinet and the Flexible Sterilization Bag during the First 3 Hours

Comparison of Location	Temperature (°C)
Average temperature in the sterilizer cabinet (set at 50°C)	50.4
Average temperature in the flexible sterilization bag	49.4
Average difference between high and low locations within the flexible sterilization bag over the 16-hour cycle	0.2
Maximum average difference within the sterilization bag	0.7
Difference between sterilizer cabinet set-point and the average temperature within the flexible sterilization bag	0.6

Table 1. Summary Statistics: Temperature in the Cabinet and in the Flexible Sterilization Bag

As noted in Table 1, the average temperature in the sterilizer cabinet and in the flexible sterilization bag differs by only 0.6°C over the entire 16-hour cycle. Within the sterilization bag itself, the average difference in temperature between the high and low locations is only 0.2°C.

### Homogeneous EO distribution

Bag 1 (impermeable bag containing orthopedic screws), Bag 2 (permeable bag containing sutures), and Bag 3 (empty impermeable sterilization bag with a frame) all had a homogeneous EO concentration within the flexible sterilization bag after the 90-minute EO vaporization phase.

The EO concentration of samples removed from Bag 1 (measured using gas chromatography), from all four corners of the sterilization bag, both above the product and deep within the product, yielded an average difference from the mean of between 0.3% and 0.7%, with a maximum relative standard deviation (RSD) of 0.8 (Table 2).

A slight reduction in EO concentration (approximately 4.1%) is evident between Group 1 (sampled at hour 1) and Groups 2 and 3 (sampled at hours 2 and 3) (Figure 9). This reduction is a direct result of EO diffusing into the product load over the first three hours. This phenomenon prevents the analysis of all groups simultaneously, so any statistical analysis was performed within sample groups only.

The distances travelled for the 11 dosimeters placed throughout Bag 1 are shown in Table 3.

EO samples removed from Bag 2 (using the gas chromatograph) yielded an average difference from the mean of between 1.1% and 1.8%, with a maximum RSD of 1.8% (Table 4).

The progressive decline in EO concentration among Groups 1, 2, and 3 is a direct result of product and package EO absorption and EO diffusion across the flexible permeable sterilization bag. As in the case of Bag 1, statistical analysis for Bag 2 (Figure 10) was performed within sample groups only.

The distance travelled for the 13 dosimeters placed throughout Bag 2 is shown in Table 5.

EO samples removed from Bag 3 (using the gas chromatograph) yielded a static EO

Dosimeter Location	Distance Travelled (mm)
1	35
2	32
3	34
4	33
5	33
6	33
7	35
8	33
9	36
10	37
11	34
Average	34
Standard deviation	1.5

**Table 3.** Results for Dosimeters Placed Throughout Bag 1 (Distance Travelled in mm). Dosimeters were removed after approximately 4.5 hours of exposure.

Dosimeter Location	Distance Travelled (mm)
1	27
2	29
3	28
4	26
5	30
6	27
7	28
8	27
9	29
10	30
11	28
12	30
13	27
Average	28
Standard Deviation	1.4

**Table 5.** Results for Dosimeters Placed Throughout Bag 2 (Distance Travelled in mm). Dosimeters were removed after approximately 4.5 hours of exposure.

concentration; there was no product to dilute the concentration or bag permeability to allow EO diffusion across the sterilization bag. This static concentration allows a comparison of all groups within the same sterilization bag. The average difference from the mean ranged from 0.2% to 1.7%, with a maximum RSD of 1.2% (Table 6). EO concentrations among Groups 1, 2, and 3 are shown in Figure 11.

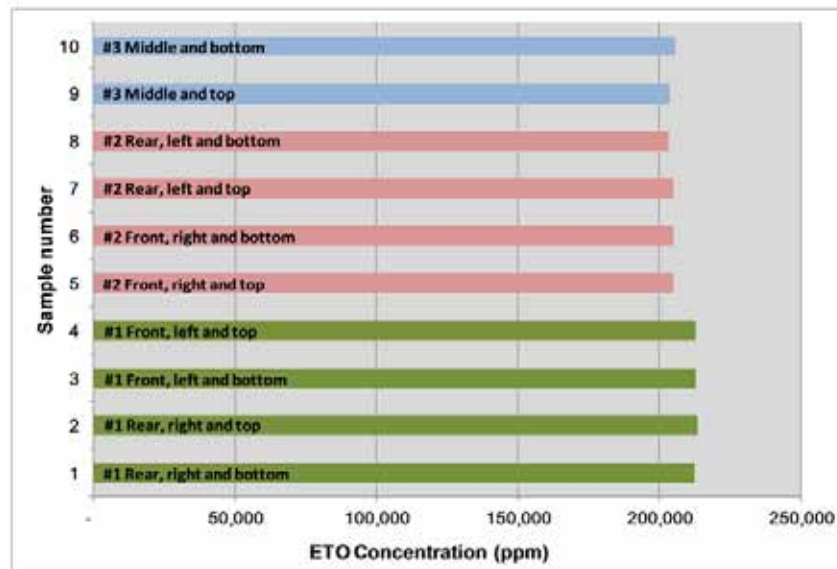
### Conclusions

#### Temperature distribution

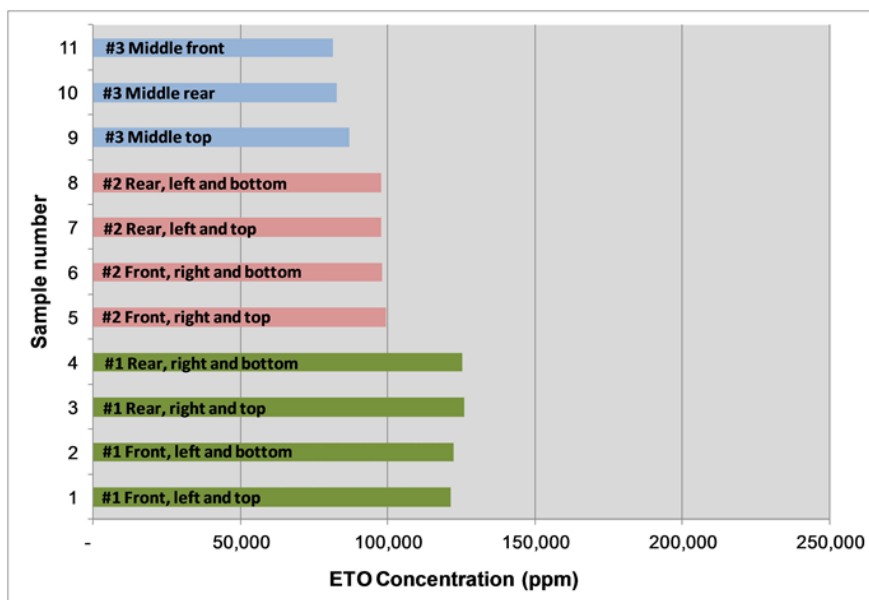
Once the temperature within the sterilization bag equilibrates with the sterilization cabinet, there is no significant temperature difference between the sterilizer cabinet and the inside of the bag; on average, this difference is only 0.6°C over the full 16-hour cycle. This 0.6°C difference is caused by the cooler thermal mass of the sterilization load. The final temperature shown by all data loggers reaches a plateau once the temperature of the sterilization load equilibrates with the sterilization cabinet. Furthermore, the 10 data

Sample Locations	Range of EO Concentration (mg/L)	Maximum Difference from the Mean	% RSD
Bag 1 Group 1	352 – 354	0.3%	0.2%
Bag 1 Group 2	337 – 340	0.7%	0.4%
Bag 1 Group 3	337 – 341	0.6%	0.8%

**Table 2.** Flexible Sterilization Bag 1: EO Concentration for Each Group



**Figure 9.** Flexible Sterilization Bag 1: Relative EO Concentration in ppm (Groups 1, 2, and 3)



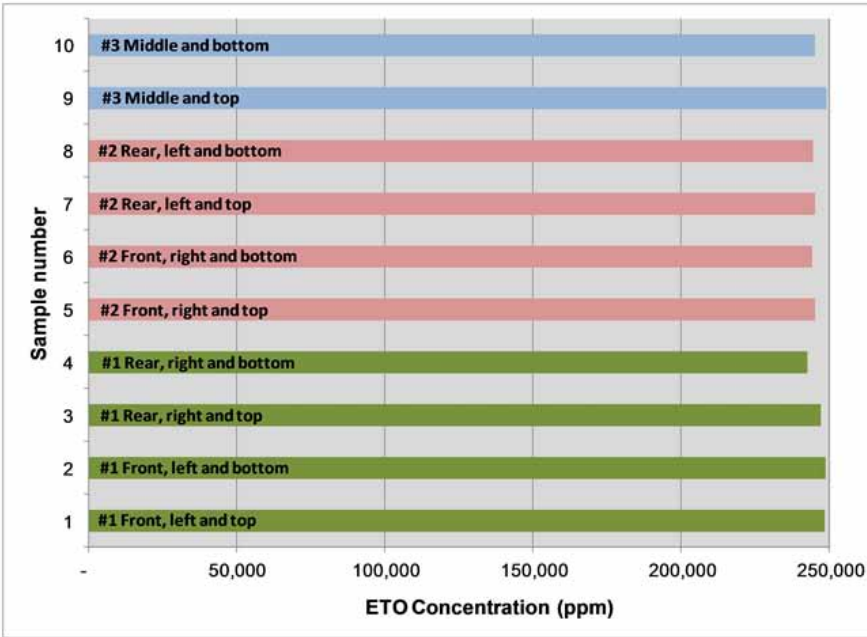
**Figure 10.** Flexible Sterilization Bag 2: Relative EO Concentration in ppm (Groups 1, 2, and 3)

Sample Locations	Range of EO Concentration (mg/L)	Maximum Difference from the Mean	% RSD
Bag 2 Group 1	201 - 208	1.8%	1.8%
Bag 2 Group 2	162 - 165	1.4%	0.9%
Bag 2 Group 3	134 - 137	1.1%	1.6%

**Table 4.** Flexible Sterilization Bag 2: EO Concentration for Each Group

Sample Locations	Range of EO Concentration (mg/L)	Maximum Difference from the Mean	% RSD
Bag 3 Group 1	402 – 412	1.7%	1.1%
Bag 3 Group 2	405 – 406	0.2%	0.1%
Bag 3 Group 3	406 – 413	0.9%	1.2%
Overall RSD for Groups 1, 2 and 3			0.9%

**Table 6.** Flexible Sterilization Bag 3: EO Concentration for Each Group



**Figure 11.** Flexible Sterilization Bag 3: EO Concentration (Groups 1, 2, and 3)

loggers distributed throughout the sterilization bag demonstrate that there is no difference in temperature in the sterilization load, whether in the corner, the center, the top, or the lower part of the load; the average difference was only 0.7°C. The maximum average difference between the highest and lowest part of the load was 0.2°C.

In a properly profiled sterilization cabinet, the powerful heaters in the sterilizer, the fans distributing the heat, and the heat-permeable sterilization bag allow the sterilization load to heat up quickly and uniformly and maintain the necessary temperature to sterilize the product load.

**EO Distribution**

The RSD was used to compare different measurements within the study. According to the Shimadzu GC manual, the expected RSD is useful for comparing the uncertainty between different measurements of varying

absolute magnitude. The RSD is calculated from the standard deviation and is commonly expressed as a percentage (%). The typical RSD for manual injections using an FID detector is <3%. The maximum RSD for all groups within this study was 1.8%. A low RSD demonstrates that the EO concentration is homogeneous throughout the sterilization bag.

Although the overall RSD cannot be calculated for Bags 1 and 2 because of concentration fluctuation, Bag 3 presents a stable, constant sterilization environment in which all samples from each group can be analyzed together. The overall RSD for Bag 3 was calculated to be 0.9%, indicating that the EO concentration is homogeneous throughout the sterilization bag, irrespective of time.

Comparing samples closest to and farthest away from the EO source verifies that high or low concentration microenvironments do not exist simultaneously (Table 6).

The dosimeters yield a similar picture and support the gas chromatography data. Dosimeter lots are calibrated with an expected standard deviation of < 2 mm, and the dosimeters from both Bag 1 and Bag 2 yielded standard deviations of 1.5 and 1.4 mm, respectively. This finding illustrates how the dosimeters are particularly useful in demonstrating EO penetration throughout the devices and device packaging.

The simplicity of the flexible sterilization bag system and the small size of the sterilization bag, coupled with the lack of any physical barriers to EO movement (other than a medical device pouch designed to allow ingress and egress of EO), allow for an unimpeded system in which the highly active EO molecules can infiltrate all areas of the sterilization bag with ease. Whether one relies on the dosimeter data or the gas chromatography data, it is clear that the EO is evenly distributed (or homogenous) throughout the sterilization bag once the EO has fully vaporized from the cartridge. The homogeneity of the ethylene oxide in the flexible chamber EO system ensures that the entire sterilization load is exposed to the identical sterilization environment, resulting in predictable sterilization cycle lethality.