

# Incidence of residual biologic debris and contamination of reused bipolar vessel sealing devices after ethylene oxide sterilization following splenectomy

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## Abstract

**Objective:** The aim of the present study was to quantify the amount of biologic debris present within disposable bipolar vessel sealing devices after each use for canine splenectomies and determine the aerobic bacterial load of the debris following instrument resterilization with ethylene oxide.

**Study design:** Prospective observational clinical study.

**Study population:** Client-owned dogs ( $n = 40$ ) presenting to a single specialty hospital for open, routine, or emergency splenectomies.

**Methods:** A total of 16 bipolar vessel sealing devices were randomly assigned to undergo one, two, three, or four splenectomies, manual hand cleanings, and ethylene oxide sterilizations before being dismantled. After final use and sterilization, each handset was agitated in phosphate-buffered saline before disassembly, which was submitted for aerobic culture. Following aseptic disassembly, all biological residue was photo-documented, collected, quantified using a subjective scoring system, and submitted for culture.

**Results:** Biologic debris was present within the inner mechanics of all devices, specifically under the blade used for vessel transection. A linear increase in debris was not appreciated amongst devices used once versus devices used multiple times. None of the devices nor any of the biologic debris cultured positive following sterilization with ethylene oxide.

**Conclusion:** The presence of biologic debris was documented after the initial use of disposable bipolar vessel sealing devices, but no devices or debris yielded positive culture results following ethylene oxide sterilization after splenectomies.

**Clinical significance:** Increased risk of iatrogenic surgical site contamination from reused vessel sealing devices is unlikely when they have been cleaned and sterilized with ethylene oxide after up to four splenectomy surgeries.

## 1 | INTRODUCTION

Bipolar vessel sealing devices (VSD) are commonly used in veterinary and human surgery during open and laparoscopic procedures to aid in more efficient dissection and decreased blood loss.<sup>1–7</sup> These devices are typically marketed as “disposable” or single-use; as such, they are generally made of less durable materials, and their intended functionality may be decreased or degraded when resterilized and reused.<sup>8–10</sup> In veterinary medicine, single-use devices (SUD) are routinely used multiple times due to the high costs associated with the equipment. While numerous studies have reviewed the ability of different disposable VSDs to perform effectively following repeated sterilization using various techniques,<sup>11–13</sup> no veterinary studies have documented the presence of biologic debris within these devices after use or the possible effect debris may have on sterilization efficacy.

These instruments are used for various clinical cases, including clean surgeries such as routine splenectomies and dirty procedures such as septic peritonitis. Residual biologic debris, defined as congealed or dried fat, blood or connective tissue, following cleaning and sterilization of reusable surgical instruments is commonly described in human literature with multiple reports of active biologic debris (or positive aerobic or anaerobic bacterial culture) and bioburden transfer between patients.<sup>9,10,14–19</sup> Contamination and bioburden transfer from reusable instruments have been identified after clean orthopedic procedures illustrating that instrumentation may harbor biological elements such as carbohydrate, protein and hemoglobin which acts as a medium for bacterial growth from other sources (skin, personnel, environment).<sup>14,17</sup> Ensuring that the debris, if present, within the reused VSDs is not biologically active is essential. A study by Saito et al. documented contamination of instruments during clean procedures (abdominal aortic aneurysm repair) with gram-positive bacteria even though the tested instrumentation (forceps) should not have encountered these microbes.<sup>20</sup> Testing instruments used in clean procedures should allow better determination of whether biologic debris becomes contaminated intra or postoperatively compared with clean-contaminated, contaminated or dirty procedures.

While most VSDs retain appropriate function between 7 and 15 uses and subsequent cleanings, only a few studies have directly assessed the effectiveness of the sterilization method in decontaminating the devices appropriately.<sup>11–13,21</sup> A recent study investigated the performance and microbiological safety of a specific VSD (LigaSure 5-mm Maryland jaw device, Medtronic Covidien) after multiple applications and resterilizations using cadaveric tissues.<sup>11</sup> The authors used hydrogen

peroxide gas sterilization and found that the instruments continued to maintain an effective seal across porcine carotid arteries up to eight sterilization cycles without the risk of bacterial contamination. Ethylene oxide (EtO) is another common means of sterilization and decontamination used for surgical instruments in private practice and university settings. While its ability to sterilize disposable vessel sealing devices without impeding function after multiple uses in cadaveric samples has been demonstrated,<sup>21</sup> the microbiological safety after reuse in live patients has not been investigated in veterinary medicine.

The main objectives of this study were to document the number of uses before biologic debris was found within the inner mechanism of the vessel sealing devices following splenectomy using client-owned dogs and to determine if the debris present was biologically active (positive bacterial culture) after multiple EtO sterilizations. We hypothesized that biologic debris would be found within the device after cleaning following the first surgical procedure but that the debris present within the devices would not be biologically active following EtO sterilization.

## 2 | MATERIALS AND METHODS

The current study was designed as a prospective observational clinical study. A total of 40 dogs presenting to a single academic teaching hospital were included for routine or emergency splenectomies between June 2021 and May 2022. The study was awarded an exemption by the Institutional Animal Care and Use Committee at the Cornell University Hospital for Animals (protocol ID no. 020221-03) as the VSDs used in this study are customarily used to perform splenectomies in the veterinary hospital. Thus, client acknowledgment prior to patient enrollment in the study was not required in addition to consent for surgery. Demographic data on the patients was collected including signalment and weight. All splenectomies were performed by either a Diplomate of the American College of Veterinary Surgeons (ACVS) or an ACVS resident via a midline celiotomy using one of the 16 new, randomly assigned single-use disposable bipolar vessel sealing devices (LigaSure 20-cm shaft Atlas, Covidien Medtronic) connected to a generator (ForceTriad, Covidien Medtronic) as previously described.<sup>22</sup> These devices were only used for the splenectomy. If a VSD was required for a subsequent abdominal procedure in the same patient, a VSD unrelated to the study was used to limit variability. If an assigned device failed in function (e.g., inability to create a seal, transect vessels, power on), the handpiece was immediately withdrawn from testing.

Intraoperative data collected included the number of energy activations used for each splenectomy, the weight of the spleens in grams, the perisplenic fat score (0 = no fat, 1 = small amount of fat, 2 = moderate amount of fat, and 3 = large amount of fat),<sup>23–25</sup> whether the splenectomy was performed for an incidental finding, presence of a hemoperitoneum, and if omental adhesions to the spleen were present. All pre-, intra-, and postoperative care was tailored to the patient's individual need, consistent with standards of practice and was not recorded for the study.

A random number generator was used to evenly assign 16 devices to groups in which one, two, three, or four splenectomy procedures, subsequent hand cleanings, and EtO sterilizations were performed. After the splenectomy was performed using the assigned VSD, the handset was cleaned with an initial wash in tap water with 2% enzymatic cleaner solution (Prolystica 2X, Steris) using a closed sink to remove all grossly visible debris and blood.<sup>11</sup> The jaws of the handset were then soaked in 2% enzymatic cleaner solution for 5 min, followed by a scrub with a sponge and metal brush using tap water with 2% enzymatic cleaner solution until gross debris was no longer visualized. That step varied in time depending upon debris burden. Devices were then air-dried and double-wrapped in sterilization wraps (Cardinal Health Sterilization Wrap, Cardinal Health) followed by placement into an ethylene oxide sterilization bag, including an ethylene oxide ampoule (AnProlene Sterilizing Bag and Gas Ampoules, Anderson Sterilizers). The wrapped devices were then exposed to ethylene oxide (AN74i Anprolene gas sterilizer, Andersen Sterilizers) for 12 h using a previously described protocol<sup>8</sup> at 50°C and >30% relative humidity as determined by a humidity chip (Humidichip, Andersen Products) and a dosimeter (Dosimeter®, Anderson Products). Sterilization indicators (3 M ethylene oxide indicator strips, 3 M Health Care) were placed within the inner wrap and on the outside of the outer wrap to ensure sterile conditions were attained. Following EtO sterilization, all devices were not used or dismantled for at least 48 h to limit and avoid potential exposure to toxic substances. The sterilized handsets were then stored in preparation to undergo the next cycle of use and testing. All devices were used within a 60-day time period and not stored beyond that length per manufacturer recommendations.<sup>26</sup>

After each device had undergone its final use, hand cleaning, and subsequent sterilization, the devices were cultured using a previously described method.<sup>11</sup> Following sterilization, the devices were aseptically removed from the sterilization wraps and placed into a sterile bowl. A total of 100 mL of phosphate-buffered saline (PBS) was introduced into each bowl containing a single device. Each device was then manually agitated in the

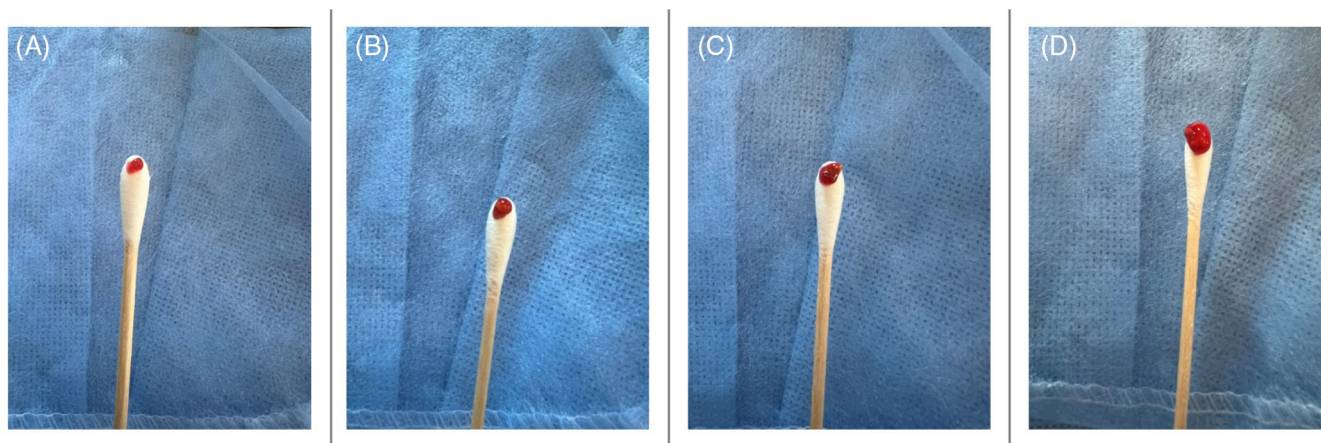
PBS for 3 min. The entire volume of PBS was then removed with a serological pipette and centrifuged for 5 min at  $3901 \times g$ . The pellet was then resuspended in 1 mL of PBS, and 100  $\mu$ L of the PBS was inoculated onto a trypticase soy agar with 5% sheep blood and incubated at 37°C for 48 h, with assessment at 24 and 48 h. After incubation, any visible bacterial colonies were counted and identified by mass spectrometry.

Following culture, each device was aseptically disassembled to assess all individual parts, and any debris present was photo-documented, collected, and submitted for aerobic and anaerobic cultures using sterile cotton-tipped swabs (Sterile Cotton Tipped Applicators, Dynarex) and anaerobic collection and transport medium (BD Port-A-Cul Vial, BD Medical) using an established standard operating procedure by the hospital's bacteriology laboratory with the specific transport medium utilized. The presence of debris was recorded as being either present or absent. If present, the volume of debris was subjectively quantified by one unblinded observer (CF) as scant, mild, moderate, or severe (Figure 1) and listed as either being within the portion of the device in contact with the surgeon, labeled as the "handpiece" of the device, or the portion of the device in contact with the patient, labeled as the "jaws" of the device (Figure 2). If a breach in sterility was recognized during any step of processing, it was directly noted.

To definitively determine whether positive bacterial growth was coming from the devices or biologic debris instead of potential background contamination, positive and negative controls were performed every fourth cycle of EtO sterilization using a previously established method.<sup>11</sup> For the negative control, 100 mL of PBS were introduced into an empty, sterilized bowl, sampled, and processed as described above before introducing a VSD into the bowl. For the positive control, 100 mL of PBS were introduced into an empty, sterilized bowl. Once the solution had been removed and formed into a resuspended pellet, it was split into two 0.5 mL samples, with one sample being inoculated with 1  $\mu$ L of *Pseudomonas aeruginosa* at  $1 \times 10^8$  organisms/mL and the other with 1  $\mu$ L of *Staphylococcus epidermis* at  $1 \times 10^8$  organisms/mL. Following bacterial inoculation, the PBS samples were incubated for 2 h before being plated for culture identification as previously described.

## 2.1 | Statistical analysis

A power analysis was performed to determine the sample size of splenectomies ( $n = 40$ ) to be performed during this study. This calculation was performed with an  $\alpha$  value of 0.05, a  $\beta$  value of 0.20, an incidence of 8.7% for the known population of surgical site infections following



**FIGURE 1** Photograph of the subjective score used for assessing the amount of debris removed from the inner mechanism of vessel sealing devices (VSD). (A) scant, (B) mild, (C) moderate, (D) severe.



**FIGURE 2** Photograph of a vessel sealing device (Ligasure Atlas, Medtronic Covidien) used in the current study after being completely disassembled into individual pieces and separated by the pieces associated with the handpiece (pieces within the white square) and the pieces related to the jaws of the device (pieces within the black rectangles). Each individual piece was then thoroughly assessed for the presence or absence of biologic debris following use in live clinical dogs for an assigned number of splenectomies.

open abdominal procedures,<sup>27</sup> and an anticipated incidence of 0% of the treated devices exhibiting positive cultures in the current study group. The number of VSDs ( $n = 16$ ) needed for the study was determined using a Fisher's exact test showing that four devices would be necessary for each group ( $n = 4$  groups) to establish significance. Categorical data are expressed as frequencies and percentages. Numerical data are reported as mean  $\pm$  standard deviation when data was normally distributed and as median and range when not normally distributed. Normality of data was determined by a Shapiro–Wilk test.

### 3 | RESULTS

#### 3.1 | Animals

A total of 40 client-owned dogs undergoing a splenectomy using study-assigned bipolar vessel sealing devices were included.

#### 3.2 | Perioperative data

A median of 23 energy activations (range: 11–45 activations) for complete ligation and transection of the splenic vessels along the hilus was recorded per splenectomy procedure. The mean  $\pm$  SD perisplenic fat score (PFS) was  $2.3 \pm 0.6$  PFS, and the median splenic weight was 520 g (range: 29–2010 g). A total of 31 dogs (77.5%) had splenectomies performed for splenic masses, 19 (19/31, 61%) of which were diagnosed with a hemoperitoneum at the time of surgery, and 22 (22/31, 70%) of which had masses adhered to the surrounding omentum necessitating increased device use within omental tissue in order to complete the splenectomy. Seven (17.5%) splenectomies were performed due to splenic congestion and vessel tearing secondary to gastric dilation and volvulus, with the remaining two (5%) splenectomies performed for immune-mediated thrombocytopenia.

#### 3.3 | Device data

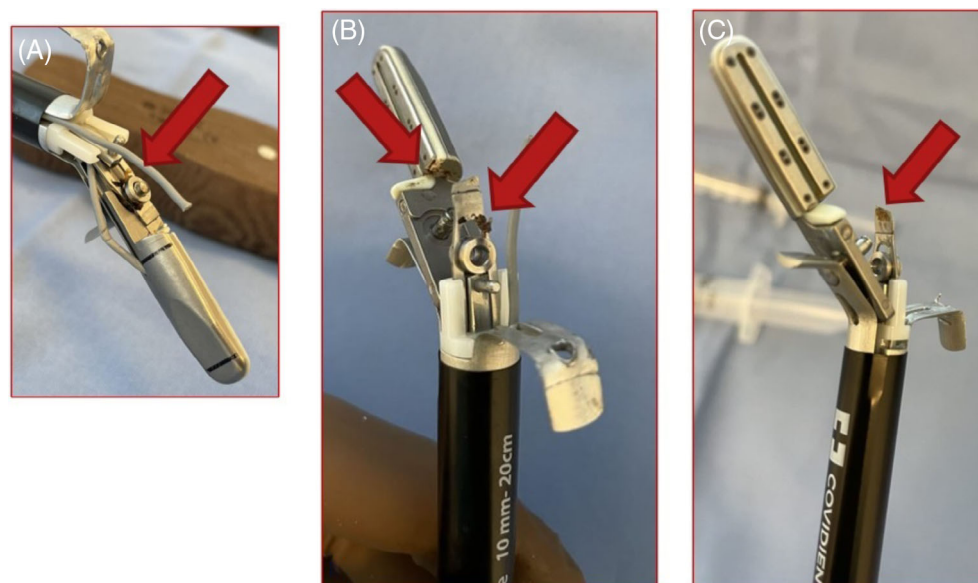
All 16 bipolar vessel sealing devices were used for 40 splenectomies (Table 1). One device exhibited a mechanical failure during its first energy activation, so it was withdrawn from the study and replaced with a new device.

All other devices successfully cycled for the remainder of the study regardless of sterilization number. All devices (16/16) exhibited biologic debris within their inner mechanics following mechanical handwashing and EtO sterilization regardless of the number of splenectomies performed, specifically within the jaws of the device (16/16) directly beneath the blade used for

transection (Figure 3). The debris was characterized as scant (14/16 devices) to mild (2/16 devices) in the tested devices. There was no debris present within any of the device handpieces. None of the devices nor the biologic debris within the devices exhibited a positive culture following EtO sterilization. All positive controls exhibited positive bacteriologic culture results.

**TABLE 1** Presence, location, and culture results of biologic debris for vessel sealing devices.

| Group                         | Ligasure number | Splenectomies performed | Debris present | Location | Debris amount | Device culture | Debris culture |
|-------------------------------|-----------------|-------------------------|----------------|----------|---------------|----------------|----------------|
| One splenectomy               | 1               | 1                       | Yes            | Jaw      | Scant         | Negative       | Negative       |
|                               | 2               | 1                       | Yes            | Jaw      | Scant         | Negative       | Negative       |
|                               | 3               | 1                       | Yes            | Jaw      | Scant         | Negative       | Negative       |
|                               | 4               | 1                       | Yes            | Jaw      | Scant         | Negative       | Negative       |
| Two splenectomies             | 5               | 2                       | Yes            | Jaw      | Scant         | Negative       | Negative       |
|                               | 6               | 2                       | Yes            | Jaw      | Scant         | Negative       | Negative       |
|                               | 7               | 2                       | Yes            | Jaw      | Scant         | Negative       | Negative       |
|                               | 8               | 2                       | Yes            | Jaw      | Scant         | Negative       | Negative       |
| Three splenectomies           | 9               | 3                       | Yes            | Jaw      | Scant         | Negative       | Negative       |
|                               | 10              | 3                       | Yes            | Jaw      | Scant         | Negative       | Negative       |
|                               | 11              | 3                       | Yes            | Jaw      | Mild          | Negative       | Negative       |
|                               | 12              | 3                       | Yes            | Jaw      | Scant         | Negative       | Negative       |
| Four splenectomies            | 13              | 4                       | Yes            | Jaw      | Mild          | Negative       | Negative       |
|                               | 14              | 4                       | Yes            | Jaw      | Scant         | Negative       | Negative       |
|                               | 15              | 4                       | Yes            | Jaw      | Scant         | Negative       | Negative       |
|                               | 16              | 4                       | Yes            | Jaw      | Scant         | Negative       | Negative       |
| Total number of splenectomies |                 | 40                      |                |          |               |                |                |



**FIGURE 3** Photograph of the jaws of the vessel sealing device (Ligasure Atlas, Medtronic Covidien) partially disassembled following manual handwashing and ethylene oxide sterilization showing the presence of biologic debris (solid red arrows) present underneath the blade used for transection after (A) one use, (B) two uses, and (C) three uses.

## 4 | DISCUSSION

The present study supports the safe reuse of VSDs following multiple use-sterilization cycles in live client-owned dogs despite the presence of biological residue within the devices even after a single procedure, allowing us to accept our hypothesis. Biologic debris was documented in each VSD after the first procedure, but the amount was subjectively scored as scant to mild and it was only found under the cutting mechanism in the jaw. This mechanism is in direct contact with tissue during sealing and dividing and it is not surprising that residue could be concealed from external sanitation procedures. The amount of debris could be expected to increase with number of uses as was seen in two devices (one after 3 uses, one after 4 uses) in the study but the volume was still too small to objectively quantify (weigh). Future studies evaluating the amount of debris present after continued use, possibly to failure, could be considered to investigate whether a linear relationship exists.

While ethylene oxide use has been documented to create toxic byproducts and many universities and practices have transitioned to more environmentally friendly alternative techniques for sterilization, it remains a commonly used sterilization method by many veterinary hospitals. Its continued use is attributed to its compatibility with most medical materials and ability to penetrate and sterilize complex medical devices, making it the ideal sterilization medium for the present study. Several veterinary studies have documented the performance of disposable, “single-use” surgical devices following repeated application and sterilization in experimental models or cadaveric tissue.<sup>11–13,21,28</sup> Our data also demonstrated the ability of this device to maintain appropriate functionality with biologic debris present when used in live dogs after four resterilization cycles with EtO. While the practice of reusing “single-use” devices has become standard in veterinary medicine, similar practices using these devices for humans remain controversial due to the concern that reuse may increase the risk of infection for patients.<sup>9,10,19,29,30</sup>

In the human literature, decontamination of reusable medical instruments with EtO following use in live patients has shown mixed efficacy. One study evaluated the effectiveness of EtO gas versus steam autoclaving for reusable endoscopic biopsy forceps.<sup>16</sup> In that study, positive bacterial cultures of *Escherichia coli* were found in 3.3% of reusable (<20 times) endoscopic biopsy forceps sterilized with EtO, concluding that EtO might be an inadequate sterilization method for biopsy forceps. One theory for the positive bacterial cultures was the instrument's complicated jaw structure interfered with the sterilization process.<sup>16</sup> The complexity of the VSD's inner

mechanism is also high, creating regions for debris to collect where decontamination cannot reach. When reviewing reports of three separate outbreaks of microbial pathogens following endoscopic and arthroscopic procedures,<sup>16–18</sup> despite the use of reusable medical instruments (endoscopic, arthroscopic forceps), clean operating rooms, and confirmed culture-negative washer-disinfectors, it was not until the lumens, handpieces, and channels of these instruments were thoroughly flushed, that biologically active debris was revealed. These studies concluded that the tissue residuals in the instruments protected the bacteria from their respective sterilizations, acting like a biofilm. This prompted the investigation of the inner mechanics of the devices used in the current study. Even during clean procedures, instrument contamination has been documented<sup>17,20</sup>; therefore, tissue debris collected during these procedures can become biologically active if contaminated at some point.

Fortunately, despite the complex construction of the VSDs, neither the devices nor the debris within the jaw blade cultured positively. The presence of biologic debris within the inner mechanics of the devices in the present study was expected but had not been previously documented in the veterinary literature. Human studies have shown inadequate removal of biological material in up to 100% of contaminated SUDs following cleaning and sterilization.<sup>10,19</sup> In those reports, it was revealed that effective disinfection eliminates microbial organisms within the present debris. Appreciating that biologic debris can be present and may become contaminated with bacteria during the course of a procedure, even a clean procedure, is essential and should be considered in surgical site infection surveillance programs. Patients undergoing surgical procedures in which SUDs are reused regularly may still have a higher risk of infection because of incomplete decontamination of such devices.<sup>9,10,19,30</sup> The ability of various sterilization mediums to eliminate different variations of pathogenic bacteria within biologic debris warrants further studies to determine the true “biofilm-like” activity this material could possess.

Limitations to the present study do exist. The results supported that sterilizing VSDs with EtO following reuse for up to four clean surgical procedures in live dogs was effective and did not result in device failure. Limiting the number of applications to four may have underestimated the effects of biologic debris on culture results and sterilization efficacy. Therefore, future studies should continue to monitor biologic debris culture results at the point when the functionality of the device ceases. While none of the devices nor the debris present within them exhibited positive cultures following sterilization, the presence of biological activity just prior to sterilization was not assessed. Due to the location of the debris within the jaw

mechanism, it would not be possible to retrieve some of the debris for a presterilization culture without completely disassembling the VSD, which would have rendered sterilization of the device inaccurate. An additional confounding factor regarding the biologic activity of the debris is the degree of heat that is generated from and through the jaws of the device which can range from 86 to 96°C (186.8–204°F).<sup>31,32</sup> While this temperature may be high enough to inactivate certain microorganisms, it is recognized that temperatures need to reach 121 or 132°C (250 or 270°F) for a prolonged period of 30 or 40 min, respectively, to kill microorganisms when utilizing steam sterilization. While not determined definitively, it is assumed that complete sterilization of the jaws and debris covering the blade could not occur from the generation of heat alone. Fortunately, the quantity of biologic debris was minute in these devices after multiple uses in clinical dogs undergoing a clean surgical procedure, but future studies should investigate the activity and amount of debris in different vessel sealing instruments, utilizing different jaw configurations and various procedures as results may vary. Lastly, the use and efficacy of EtO for the reesterilization of these devices and the biologic debris within the inner mechanics when used for clean-contaminated, contaminated, and dirty procedures should be performed in future studies.

In conclusion, the presence of biologic debris was documented within the inner mechanics of the vessel sealing devices following completion of a single clean procedure; however, the debris was inactive following ethylene oxide sterilization for up to four uses. Further studies are indicated to determine whether the debris within these devices after clean-contaminated or contaminated procedures is also inactive and whether this debris is associated with complications such as patient infections or equipment malfunction.

## AUTHOR CONTRIBUTIONS

Folk CA, DVM: Substantial contribution to the acquisition of data, data analysis, interpretation of data, drafting the manuscript, revising the manuscript, and approving the final version of the manuscript to be published. Buote NJ, DVM, DACVS (Small Animal): Substantial contribution to the conception of the study design, acquisition of data, survey data analysis, interpretation of survey data, revising the manuscript, and approving the final version of the manuscript to be published. Socha DE, DVM: Acquisition of data, interpretation of data, revising the manuscript, and approving the final version of the manuscript. Hayes GM, PhD, DACVECC, DACVS (Small Animal): Acquisition of data, interpretation of data, revising the manuscript, and approving final version of the manuscript.

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## CONFLICT OF INTEREST STATEMENT

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