

# Evaluation of XSTAT® and QuickClot® Combat Gauze® in a Swine Model of Lethal Junctional Hemorrhage in Coagulopathic Swine

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## ABSTRACT

**Background:** Hemorrhage is associated with most potentially survivable deaths on the battlefield. Effective and field-tested products are lacking to treat junctional and noncompressible injuries. XSTAT® is a newly developed, U.S. Food and Drug Administration-approved product designed to treat junctional hemorrhage. The Committee on Tactical Combat Casualty Care has recently approved the product for use as part of its treatment guidelines, but data are lacking to assess its efficacy in different wounding patterns and physiologic states. **Methods:** Dilutional coagulopathy was induced in 19 large (70–90kg), healthy, male swine by replacing 60% of each animal's estimated blood volume with room temperature Hex-tend®. After dissection, isolation, and lidocaine incubation, uncontrolled hemorrhage was initiated by transection of both axillary artery and vein. Free bleeding was allowed to proceed for 30 seconds until intervention with either XSTAT or QuickClot® Combat Gauze® (CG) followed by standard backing. Primary outcomes were survival, hemostasis, and blood loss. **Results:** XSTAT-treated animals achieved hemostasis in less time and remained hemostatic longer than those treated with CG. Less blood was lost during the first 10 minutes after injury in the XSTAT group than the CG group. However, no differences in survival were observed between XSTAT-treated and CG-treated groups. All animals died before the end of the observation period except one in the XSTAT-treated group. **Conclusion:** XSTAT performed better than CG in this model of junctional hemorrhage in coagulopathic animals. Continued testing and evaluation of XSTAT should be performed to optimize application and determine appropriate indications for use.

**KEYWORDS:** XSTAT; trauma; hemorrhage; hemorrhage, junctional; Combat Casualty Care

## Introduction

Traumatic hemorrhage, particularly when occurring on the torso, is responsible for the greatest number of potentially survivable deaths in recent conflicts.<sup>1,2</sup> Fast, effective, and easily applied treatments for junctional and noncompressible hemorrhage are needed because treating these injuries is challenging with current standards of care. Junctional tourniquets and resuscitative endovascular balloon occlusion of the aorta (REBOA) are promising new technologies.<sup>3–6</sup> However,

each has limitations that make it insufficient for some injury locations or wounding patterns. Junctional tourniquets are mainly effective when injuries are slightly distal to the junction,<sup>7</sup> whereas REBOA is more complex to implement and does not work for injuries of the upper torso.<sup>8</sup> Hemostatic gauzes, including QuikClot® Combat Gauze (CG; Z-Medica, www.z-medica.com), are effective in controlling hemorrhage in compressible sites but do not offer definitive hemostasis.<sup>9,10</sup> Therefore, the development and testing of new products in different wounding patterns and physiological states will improve point-of-care treatments.

XSTAT® (RevMedx, <http://www.revmedx.com>) is a newly developed hemostatic device designed to treat junctional wounds in the groin or axilla by the injection of self-expanding, mini sponges directly into a bleeding wound. The device has been approved by the Food and Drug Administration and is recommended by the Committee on Tactical Combat Casualty Care (CoTCCC) to control bleeding.<sup>11,12</sup> It has been shown to be more effective than CG in a swine model of junctional hemorrhage.<sup>13,14</sup> XSTAT also is applied significantly faster than standard gauze and produced pressure throughout a wound cavity in a simulated injury using ballistic gel.<sup>15</sup>

The aim of this study was to compare XSTAT with CG, one of the CoTCCC standards of care for hemostatic dressings. A junctional injury was created in coagulopathic swine before application of the test dressing. We hypothesized that XSTAT would be more effective in creating hemostasis, because it relies less on coagulation factors and because it produces even pressure throughout a wound.

## Materials and Methods

### Overview

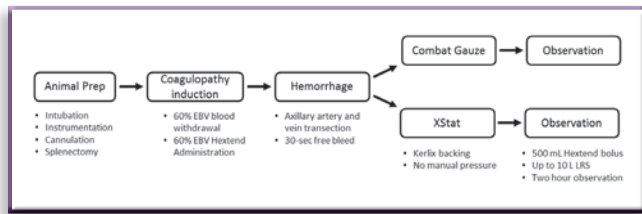
This was a randomized, blinded, prospective trial. Male, Yorkshire–Landrace swine, weighing 70–90kg were used in the experimental protocol. All subjects were treated according to “The Guide for the Care and Use of Laboratory Animals”<sup>16</sup> and the animals were housed according to regulation at an Association for Assessment and Accreditation of Laboratory Animal Care International-accredited facility. The study was approved by the U.S. Air Force 59th Medical Wing's Institutional Animal Care and Use Committee. Animals were excluded from analysis when the subject died

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before treatment or a significant deviation from protocol occurred. An overview of the experimental protocol is shown in Figure 1.

**Figure 1** Experimental schematic.



EBV, estimated blood volume; LRS, lactated Ringer's solution.

### Surgical Preparation

Animals were fasted overnight before surgery but allowed free access to water. They were sedated with 4.4mg/kg tiletamine-zolazepam intramuscularly (IM) and 2.2mg/kg ketamine IM. Buprenorphine was then given for alleviation of pain at a dose of 0.01mg/kg IM. Anesthesia was induced via mask with 2% to 4% isoflurane in an air/oxygen mixture of 40% to 60%. After intubation, isoflurane was adjusted to maintain a minimum alveolar concentration of at least 1.2.

Vascular access was obtained using a modified Seldinger technique. The left external jugular vein was accessed for resuscitation fluids, and a pulmonary artery catheter (Edwards Lifesciences, <http://www.edwards.com>) was inserted via the right external jugular vein. The right carotid artery was accessed to monitor blood pressure and to allow for blood sampling. Splenectomy was performed through a midline laparotomy to prevent splenic autoperfusion during hemorrhage, followed by a cystostomy for urine collection.

### Induction of Coagulopathy

Induction of coagulopathy was performed according to previous studies.<sup>5,17</sup> Briefly, 60% of the estimated blood volume was removed via the right femoral artery at 50mL/min. Simultaneously, room-temperature Hextend (BioTime, <http://www.biotimeinc.com>) was infused at the same rate through the right-side external jugular vein. Hypothermia was allowed to progress until a temperature of 34.5°C (94.1°F) was reached; subsequently, warming blankets were used to keep temperatures near 34.5°C (94.1°F) until the injury phase.

### Injury and Intervention

To gain access to the axillary artery and vein, a 4cm incision was made parallel to the sternum over the pectoralis major muscle. The axillary artery, axillary vein, and brachial plexus were then minimally dissected away (approximately 2cm) from the surrounding tissue. Wound cavity volume was determined by measuring the amount of warmed saline necessary to fill the wound cavity. The vessels were then bathed in 2% lidocaine for 10 minutes to induce dilation. After suction removal of lidocaine, a necropsy blade was used to transect both the axillary artery and vein to initiate injury (time = 0 minutes). Hemorrhage was allowed to proceed for 30 seconds while blood was collected by suction and weighed. The test hemostatic dressing was then applied to the wound using either a single roll of CG or up to four XSTAT applicators. Kerlix was packed into the wound as backing, but no manual compression was applied in either group. This deviation from both products' instructions for use was done to test them as equivo- cally as possible.

The time it took to pack both the test product and Kerlix backing was defined as pack time. Hemostasis was defined as no blood leaving the wound cavity, and immediate hemostasis was defined as hemostasis occurring at the end of wound packing. Duration of hemostasis is the time during the experiment when hemostasis was occurring while the animal was alive. Blood flowing from the wound was collected by suction after treatment in two phases: the initial 10 minutes (early blood loss) and the remainder of the 2-hour observation period (late blood loss).

After injury, animals were given a 500mL bolus of Hextend at 100mL/min through the left external jugular vein. Following this bolus, up to 10L of lactated Ringer's solution was administered at 100mL/min to maintain a mean arterial pressure between 60mmHg and 65mmHg, in keeping with previous similar studies.<sup>18</sup> Death was defined as a mean arterial pressure (MAP) less than 20mmHg and end-tidal carbon dioxide (EtCO<sub>2</sub>) less than 15mmHg maintained for 2 minutes. Animals were killed by an overdose of pentobarbital once death criteria were reached or when 2 hours had passed from the initial injury.

### Outcomes and Analysis

The primary outcomes used in this study were survival, hemostasis, and blood loss. Secondary outcomes included the following hemodynamic parameters: heart rate, MAP, EtCO<sub>2</sub>, cardiac output, central venous pressure, and mean pulmonary artery pressure. Metabolic factors analyzed included lactate level, base excess, pH, and, the volume of resuscitation fluids (i.e., lactated Ringer's solution) used to maintain MAP above 60mmHg.

Data are presented as mean ± standard deviation unless otherwise noted. One-way analysis of variance (ANOVA) was used for most analyses. However, Kruskal-Wallis ANOVA on ranks was used when the normality test failed (i.e., if  $p < .05$ ). Survival and hemostasis were analyzed using the Fisher exact test. Additionally, survival was analyzed by log-rank analysis. Statistical analysis and data management were performed using Excel 2010 (Microsoft, [www.microsoft.com](http://www.microsoft.com)) and Sigmaplot 12 (Systat Software, <https://systatsoftware.com>).

## Results

### Study Group Statistics

A total of 19 animals weighing 75.9kg ± 4.5kg were included for analysis in this study and randomly assigned to groups as follows: CG ( $n = 10$  animals) and XSTAT ( $n = 9$  animals). The two groups were similar at baseline with no statistically significant differences between means of each group (Table 1). Four animals had to be excluded from the analysis: one animal died during coagulopathy and was not included in randomization, one animal from the CG group and one from the XSTAT group were excluded because of a protocol deviation of 45% oxygen during injury, and one animal randomly assigned to the XSTAT group was excluded because of infusion pump failure.

### Induction of Coagulopathy

The replacement of 60% of estimated blood volume with Hextend resulted in the administration of 2,962 ± 172mL over 59.2 ± 3.5 minutes and the removal of 2,794 ± 162mL of blood. The two groups were similar after coagulopathy induction and there were no significant differences between

**Table 1** Preinjury Characteristics

| Characteristic                                | QuikClot Combat Gauze | XSTAT       | <i>p</i> Value |
|---|-----------------------|-------------|----------------|
| <b>Baseline values</b>                        |                       |             |                |
| Weight (kg)                                   | 76.3 ± 5.0            | 75.6 ± 4.1  | .745           |
| MAP (mmHg)                                    | 60.4 ± 9.2            | 62.5 ± 10.4 | .654           |
| MPAP (mmHg)                                   | 18.3 ± 1.6            | 18.7 ± 2.7  | .756           |
| CVP (mmHg)                                    | 7.4 ± 1.9             | 7.2 ± 1.6   | .862           |
| Heart rate (bpm)                              | 60.3 ± 7.2            | 55.0 ± 7.2  | .135           |
| Temperature (°C)                              | 37.3 ± 0.3            | 37.1 ± 0.7  | .710           |
| <b>Values after induction of coagulopathy</b> |                       |             |                |
| Hextend coagulopathy (mL)                     | 2,975 ± 192           | 2,947 ± 159 | .728           |
| Blood removed (mL)                            | 2,817 ± 187           | 2,852 ± 209 | .704           |
| INR   | 1.25 ± 0.05           | 1.28 ± 0.04 | .228           |
| Hemoglobin (g/dL)                             | 9.9 ± 0.6             | 10.1 ± 0.9  | .557           |
| Temperature (°C)                              | 35.2 ± 0.6            | 35.1 ± 1.0  | .841           |
| Preinjury MAP (mmHg)                          | 68.7 ± 10.1           | 66.4 ± 6.4  | .574           |
| <b>Postinjury/pretreatment values</b>         |                       |             |                |
| Pretreatment blood loss (mL)                  | 849 ± 228             | 720 ± 225   | .479           |
| MAP at end of injury (mmHg)                   | 38.9 ± 7.6            | 38.1 ± 6.4  | .538           |

CVP, central venous pressure; INR, international normalized ratio; MAP, mean arterial pressure; MPAP, mean pulmonary artery pressure.

means of each group (Table 1). Coagulopathy was observed by an increase in international normalized ratio from 1.07 ± 0.05 to 1.45 ± 0.10 second ( $p < .001$ ), and overall hemoglobin levels decreased from 10.0 ± 0.8g/dL to 4.0 ± 0.4g/dL ( $p < .001$ ). Mild hypothermia was observed as rectal temperature decreased from 37.2°C (99°F) ± 0.5°C (33°F) at baseline down to 35.2°C (95.4°F) ± 0.8°C (33.4°F;  $p < .001$ ) after induction of coagulopathy.

### Injury

Before injury, animals had a MAP of 67.6 ± 8.4mmHg with no significant differences between groups (Table 1). Cavity volumes were similar between groups, with a volume of 104 ± 15mL and 117 ± 33mL for the XSTAT and CG groups, respectively ( $p = .324$ ). After complete transection of both the axillary artery and vein, 813mL ± 220mL of blood was lost after 30 seconds of free bleeding, leading to a mean MAP of 38.5mmHg ± 6.9mmHg. The two groups were similar after injury with no significant differences between means of each group (Table 1).

### Hemostatic Dressing Performance

Hemostatic dressings were applied through the pool of blood at the wound site. Pack time, which included time for both the test dressing and Kerlix backing to absorb blood, was 16 seconds shorter with XSTAT than with CG (Table 2). The number of XSTAT applicators used varied from two to four (mean, 2.8 ± 0.8 applicator). One XSTAT applicator, of the 27 used, malfunctioned during application. The exact mechanism of failure was inconclusive and not determined to be user error or manufacturer error.

The achievement of hemostasis was considered the primary outcome. Only one animal (in the XSTAT group) had hemostasis immediately after treatment. Nearly all XSTAT-treated animals achieved an eventual hemostasis, whereas fewer than half of CG did (Table 2). Of the animals that did reach

**Table 2** Postintervention Data

| Characteristic                     | QuikClot Combat Gauze ( <i>n</i> = 10) | XSTAT ( <i>n</i> = 9) | <i>p</i> Value    |
|------------------------------------|--|-----------------------|-------------------|
| Application time (seconds)         | 87.1 ± 17.4                            | 71.5 ± 17.6           | .069              |
| Immediate hemostasis, no. (%)      | 0 (0)                                  | 1 (11)                | .474              |
| Eventual hemostasis, no. (%)       | 4 (40)                                 | 8 (89)                | .057              |
| Time to reach hemostasis (minutes) | 33.8 ± 4.8                             | 20.3 ± 9.8            | .028 <sup>a</sup> |
| Duration of hemostasis (minutes)   | 5.4 ± 9.5                              | 25.6 ± 31.3           | .029 <sup>a</sup> |
| Time to death (minutes)            | 35.4 ± 16.0                            | 48.9 ± 29.1           | .438              |
| Early blood loss (mL)              | 847 ± 665                              | 435 ± 398             | .058              |
| Late blood loss (mL)               | 312 ± 373                              | 434 ± 435             | .377              |
| Survival, no. (%)                  | 0 (0)                                  | 1 (11)                | .474              |

<sup>a</sup> $p < .05$ .

hemostasis, the time to achieve hemostasis was significantly shorter with XSTAT than with CG ( $p < .05$ ). Similarly, the duration of hemostasis (i.e., the total time animals survived while hemostatic) was also significant when comparing XSTAT with CG ( $p < .05$ ).

After completion of packing, blood was collected and weighed. This blood was separated into that collected during the first 10 minutes after packing ("platinum" 10 minutes) and that collected during the rest of the observation period (Table 2). During the platinum 10 minutes, CG-treated animals bled more than XSTAT-treated animals did, with the differences approaching significance ( $p = .058$ ). However, total blood loss over the full 2-hour observation period was not significantly different between groups.

### Discussion

This study was performed to evaluate the efficacy of XSTAT in comparison with CG in a model of junctional hemorrhage, a leading cause of potentially survivable deaths on the battlefield. The hemostatic products were tested in a lethal model of axillary arterial and venous injury in the context of adult-sized (70–90kg) swine with dilutional coagulopathy. After treatment, the animals were resuscitated with a 500mL bolus of Hextend followed by up to 10L of lactated Ringer's solution to keep the MAP between 60mmHg and 65mmHg and to follow the Department of Defense consensus model for evaluating hemostatic dressings.<sup>18</sup>

The results of this study show that XSTAT was more effective than CG in reaching and maintaining hemostasis, and had less bleeding in the first 10 minutes after application. XSTAT also had a quicker application time, confirming previous studies, but this study included both packing of backing and test dressing, masking differences between the two dressings. Despite these results, there were not any significant differences in survival or time of death.

XSTAT-treated animals had significantly less blood loss than CG-treated animals during the first 10 minutes after injury. This period, the platinum 10 minutes, was chosen a priori as an end point to illustrate differences between products during the critical period after trauma. This examination of the blood loss before any animal death offers a more complete comparison of

dressing performance without the censor of data from animal death. Additionally, this 10-minute distinction has proven effective previously in similar product evaluation trials.<sup>10</sup>

There was not a direct relationship between achievement of hemostasis and survival in the data presented here. For example, animals that had relatively early hemostasis paradoxically did not survive the full observation period. Furthermore, animals that had little bleeding after dressing application still died. In fact, the animal that bled the least died the earliest in the XSTAT group. These contradictory results imply that the coagulopathy combined with the aggressive resuscitation paradigm was partly responsible for the high mortality rates and not solely due to dressing performance.

No manual pressure or pressure dressings were used in this study. Interestingly, a study performed by Navy researchers did not find any difference with or without manual pressure in a similar model of swine axillary injury.<sup>13</sup> The instructions for XSTAT use are to “Cover the wound with an occlusive or pressure dressing. If available, use an elastic bandage. If bleeding persists, apply manual pressure until bleeding is controlled.”<sup>19</sup> The instructions for CG application are to “. . . apply pressure for 3 minutes or until bleeding stops. Wrap and tie bandage to maintain pressure.”<sup>20</sup> Standard gauze backing was used in these experiments to make the findings more generalizable to various wounding patterns.

There are limitations to this study, including the lack of a defined correlation between hemostasis and survival, as mentioned. The wound produced was surgical and likely does not reflect real-world injury patterns. However, XSTAT is designed such that the small sponges can expand into any cavity shape. Additionally, these experiments were performed in a controlled laboratory setting with relatively small sample sizes. Nevertheless, the results produced statistically different results between the two products using this junctional injury model.

Currently, XSTAT is recommended by the CoTCCC as “best for deep, narrow-tract junctional wounds.”<sup>12</sup> Future studies may aim to expand the recommendation of the CoTCCC to allow XSTAT to be applied to regions and circumstances outside junctional wounds, such as the neck, abdomen, or pelvis. The product could also be used in different situations outside the point of injury, as was seen in the first combat casualty use.<sup>11</sup> After failure to control intraoperative bleeding from a leg wound by using cautery and hemostatic gauze, XSTAT was successfully used to stop the bleeding.<sup>11</sup>

## Conclusion

The CoTCCC recently added XSTAT to its list of approved hemostatic dressings. This study confirms that recommendation and provides new evidence of XSTAT's efficacy in restoring hemostasis in a rapidly bleeding wound. XSTAT-treated animals achieved hemostasis in less time and remained hemostatic longer than those treated with CG, but these differences did not result in increased survival in this model of uncontrolled hemorrhage in swine with coagulopathy.

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## Disclaimer

The views expressed are those of the authors and do not reflect the official views of the Department of Defense or its components. The experiments reported herein were conducted according to the principles set forth in the National Institute of Health Publication No. 80-23, Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1996, as amended.

## Disclosures

The authors have indicated they have no financial relationships relevant to this article to disclose.

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