Designation: F3207 – 17

Standard Guide for *in vivo* Evaluation of Rabbit Lumbar Intertransverse Process Spinal Fusion Model[1]

This standard is issued under the fixed designation F3207; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 Historically, the single-level rabbit posterolateral, or intertransverse, lumbar spine fusion model was developed and reported on by Dr. Scott Boden, et. al. (Emory Spine Center for Orthopedics) and the model has been proposed as a non-clinical model which may be used to replicate clinically-relevant fusion rates for iliac crest autograft in the posterolateral spine (**1**, **2**).[2] This model is used routinely in submissions to regulatory bodies for the purpose of evaluating the potential efficacy of bone void filler materials as compared to other materials or iliac crest autograft to effect spinal posterolateral fusion. The use of this standard's recommendations as part of a regulatory submission does not provide any guarantee of regulatory clearance and should be considered as a part of the data provided for regulatory submission.

1.2 This guide covers general guidelines to evaluate the effectiveness of products intended to cause and/or promote bone formation in the lumbar intertransverse process spinal fusion model *in vivo*. This guide is applicable to products that may be composed of one or more of the following components: natural biomaterials (such as demineralized bone), and synthetic biomaterials (such as calcium sulfate, glycerol, and reverse phase polymeric compounds) that act as additives, fillers, and/or excipients (radioprotective agents, preservatives, and/or handling agents). It should not be assumed that products evaluated favorably using this guidance will form bone when used in a clinical setting. The primary purpose of this guide is to facilitate the equitable comparison of bone void fillers and/or autograft extender products *in vivo*. The purpose of this guide is not to exclude other established methods.

1.3 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with the use of bone void fillers. It is the responsibility of the user of this standard to establish appropriate safety and health practices involved in the development of said products in accordance with applicable regulatory guidance documents and in implementing this guide to evaluate the bone-forming/promoting capabilities of the product.*

1.5 *This standard does not purport to address the requirements under 21 CFR Part 58 concerning Good Laboratory Practices or international standard counterpart OECD Principles of Good Laboratory Practice (GLP). It is the responsibility of the sponsor of the study to understand the requirements for conduct of animal studies whereby the data may be used to support premarket applications, including requirements for personnel, protocol content, record retention and animal husbandry.*

1.6 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[3]

E122 Practice for Calculating Sample Size to Estimate, With Specified Precision, the Average for a Characteristic of a Lot or Process

E1402 Guide for Sampling Design

E1488 Guide for Statistical Procedures to Use in Developing and Applying Test Methods

F2529 Guide for ***in vivo*** Evaluation of Osteoinductive Potential for Materials Containing Demineralized Bone (DBM)

F2884 Guide for Pre-clinical ***in vivo*** Evaluation of Spinal Fusion

2.2 *Federal Documents:*[4]

21 CFR 58 Good Laboratory Practice for Nonclinical Laboratory Studies

[1] This guide is under the jurisdiction of ASTM Committee F04 on Medical and Surgical Materials and Devices and is the direct responsibility of Subcommittee F04.44 on Assessment for TEMPs.

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[2] The boldface numbers in parentheses refer to the list of references at the end of this standard.

[3] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[4] Available from U.S. Food and Drug Administration (FDA), 10903 New Hampshire Ave., Silver Spring, MD 20993, http://www.fda.gov.

2.3 *AAMI/ISO Documents:*[5]

ISO 10993-6 Third edition 2016-12-01 Biological evaluation of medical devices — Part 6: Tests for local effects after implantation

3. Terminology

3.1 *Definitions:*

3.1.1 *biomechanical fusion, n*—the increased strength and/or stiffness and reduced ROM of a spinal unit as compared to that measured before surgical intervention.

3.1.2 *biomechanical properties, n*—as used in this document, evaluation of the operative functional spinal unit multidirectional range of motion (ROM: Lateral bending, Flexion – Extension and Axial Rotation) properties under non-destructive conditions, tensile stiffness and ultimate load.

3.1.3 *fusion, n*—a multifactorial outcome which can be characterized in terms of the radiographic, biomechanical and histological results of the intended spinal arthrodesis procedure.

3.1.4 *histological evidence of fusion, n*—based on light microscopy of newly formed and remodeled bone spanning the intertransverse region, with contiguous osseous connectivity observed between the adjacent transverse processes. Assessment rationale must be justified.

3.1.5 *manual palpation, n*—a method for evaluating spinal fusion status by estimating the stiffness of the operative motion segment and adjacent superior motion segment by the application of multidirectional loads in lateral bending and flexion-extension using the hands.

3.1.6 *micro-computed tomographic (micro-CT) fusion, n*—tomographic fusion is based on interpretation of the micro-CT images, with fusion success based on the three-dimensional appearance of contiguous bone from transverse process to transverse process (i.e. bridging bone).

3.1.7 *non-union, n*—a multifactorial outcome which can be characterized in terms of the radiographic, biomechanical and histological results indicating a lack of trabecular or cortical bone spanning the intertransverse region, without contiguous osseous connectivity observed between the adjacent transverse processes.

3.1.8 *radiographic fusion, n*—status of radiographic fusion is based on interpretation of the posteroanterior (P/A) plain film x-ray images, with fusion success based on the appearance of contiguous bone from transverse process to transverse process (i.e. bridging bone).

4. Significance and Use

4.1 This guide covers animal implantation methods and analysis of bone void fillers to determine whether a material or substance leads to lumbar intertransverse process spinal fusion, as defined by its ability to cause bone to form *in vivo*.

[5] Available from Association for the Advancement of Medical Instrumentation (AAMI), 4301 N. Fairfax Dr., Suite 301, Arlington, VA 22203-1633, http://www.aami.org.

5. Animal Models

5.1 *General Note*—Appropriate positive or comparative controls may be used. For example, comparative controls could be similar devices, and positive controls could be autograft from the animal.

5.2 *Skeletally mature New Zealand white rabbits*—(typically > 7 months and 3.5-4.5kg). Proximal tibial and distal femoral physes should be closed and verified via plain radiographs. Radiographic and histologic closure of the distal femoral growth plates occur at an average age of 21 and 22 weeks, respectively. The proximal tibial physes close radiographically and histologically at an average age of 26 and 28 weeks, respectively. A lateral radiograph is a more reliable method for assessing physeal closure in the rabbit, and radiographic confirmation of tibial physeal closure should be obtained prior to using rabbits that are younger than approximately 7 months of age. Radiographic confirmation of physeal closure is probably not necessary in rabbits 8 months or older but should be provided for the sake of completeness. Some minor variation in age of tibial growth plate closure may be expected with different strains of New Zealand White rabbits. Weight is not a reliable indicator of skeletal maturity in the New Zealand White rabbit. All rabbits used should be of the same sex. **(3, 4)**

5.3 *Implant Mass/Volume*—In general, implant mass (~1.6-2.2 grams; useful only for autograft)/volume (~2.5-3.0 cc) per side is used. It is recommended that the experimental group contain the same total implant volume as any comparative groups so the results are comparable and the potential effects of the implant on intertransverse process spinal fusion can be determined.

5.4 *Sample Size:*

5.4.1 Sample sizes should be justified in the study protocol and, if possible, should provide statistical power appropriate to the endpoint using appropriate statistical methods to justify as required. Interim time points may be used as appropriate and justifications should be provided. Should statistical numbers not be practical or possible, empirical testing in the literature has shown an n=6-8 to be a target sample size minimum.

5.4.2 Sample size should be determined with reference to the primary outcome of the study, which is typically the fusion rate at 8 or 12 weeks. Additionally, it may be necessary to consider the sampling requirements of other analyses in the study; in particular, quantitative endpoints such as morphometry.

NOTE 1—The sample size recommendations refer to the number of samples expected to be available for analysis. Attrition, or loss of animals due to surgical complications, is common in the rabbit spinal fusion model (especially with autograft harvesting). It may be necessary to plan for additional animals to replace those lost to attrition. Make sure you report all animals treated, any unexpected or early deaths, etc.

5.5 *End Points*—Each implant group should have an immediate post-operative assessment and end points should be justified by the resorption profile of the materials; there should be at least 2 time points less than the maximum assessment time (an early and mid-phase) in order to assess any irregularities (unexpected or excessive inflammation, etc.) at the

implant and peri-implant site (recommended time periods representative in the literature are 4, 8, and 12 weeks) or longer periods may be warranted and should be justified.

6. Recommended Surgical Protocol Methodologies

6.1 *Rabbit Lumbar Intertransverse Process Spinal Fusion Recommended Surgical Technique:*

6.1.1 Aseptic technique should be employed during the surgical implantation procedures.

6.1.2 Animals should be singly housed in standard cages and fed with rabbit food and water.

NOTE 2—Handling of the animals during the first 14 days post-op should be avoided unless medically required.

6.1.3 Pre-operative analgesics: 0.05 mg/ kg buprenorphine administered subcutaneously and the application of a fentanyl patch (25 µg/hr) to the inner ear pinna, or other analgesic approved by the IACUC. A 25 µg/hr fentanyl patch is an effective analgesic with duration of up to 72 hours, but may require up to 12 hours after application until blood levels are sufficient to provide pain relief. Patches may be placed the evening prior to surgery or animals dosed with an analgesic such as butorphanol prior to surgery and several hours after surgery to ensure analgesisc coverage while fentanyl blood levels rise.

6.1.4 Anesthetics: Induction and maintenance: 34 mg/ kg ketamine and 5 mg/ kg xylazine administered intramuscularly. Isoflurane should be administered via laryngeal mask within a range of 2%-3%, but increasing and decreasing the percentage administered should be based on the individual animal response. Ophthalmic ointment should be applied to the eyes following pre-anesthesia and prior to surgery.

6.1.5 Identify each animal with a unique identifier (ear tag, tattoo, etc.). Record the individual animal identification numbers along with the body weights.

6.1.6 Sedate the animal with an IACUC approved medication and maintain general anesthesia with Isoflurane or other anesthetic approved by the IACUC. The depth of anesthesia should be sufficient to prevent muscular movement. This can be checked by pinching the toe (between the digits) of the animal's hind limbs. If there is a reflex reaction, the animal is not sufficiently anesthetized to continue with the implantation. A technician shall monitor the animal's vitals/parameters while under anesthesia and record every 15 minutes.

6.1.7 Place the anesthetized animal in a sternal or ventral recimbant position on a clean flat surface in a procedure room and shave the dorsum of the animal from the mid thoracic region well below the iliac crests with clippers. Scrub the clipped area with surgical scrub (chlorhexidine scrub or povidone scrub). Start from the center and work, in a circular fashion, to the edge of the surgical area. Wipe off the surgical scrub with 70 % isopropyl alcohol (repeat entire scrub procedure at least 3 times). The surgeon will complete final preparation for aseptic surgery.

6.1.8 Transfer the anesthetized animal to the surgical suite.

6.1.9 Lumbar Posterolateral Intertransverse spinal fusion is detailed as follows:

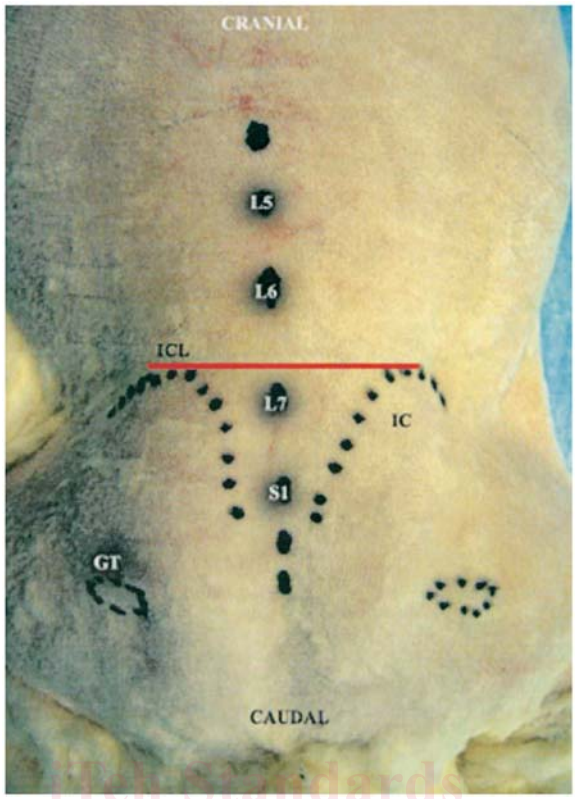
6.1.9.1 Final sterile prep of the surgical site is completed in the operating room with 2% chlorhexidine or povidone solution prior to first incision. Start from the center and work to the edge of the surgical area. Wipe off the solution with a clean, sterile gauze pad. The spinal level to be fused, most commonly L4–L5 or L5–L6, is then identified by palpation. A line drawn from the most cranial aspect of one iliac crest to the other, the intercrestal line, will generally pass between the L6 and L7 spinous processes (Fig. 1). A second method to verify the correct operative level is based on the anatomy of the lumbosacral spinous processes. Specifically, there is often a much wider interspinous distance at L6–L7 than there is at L5–L6, L7–S1 or between the sacral processes (Fig. 1), although this is not always the case.

6.1.9.2 Using both techniques of localization, the L4–L5 or L5–L6 level can be correctly identified in the vast majority of animals. Errors can occur, however, because of the presence of osseous anomalies of the lumbosacral vertebrae. A preoperative dorsoventral radiograph is advisable. In the presence of an abnormality, which alters the typical number of lumbar motion segments, the animal should be excluded or the spinal level just cranial to the intercrestal line can be used.

6.1.9.3 It is acceptable to perform the surgery at either L4–L5 or L5–L6, vertebral levels, although the choice of level should be consistent within the study. Variability in the number of lumbar vertebrae is common in certain strains of NZW rabbits-some rabbits exhibiting 6 lumbar vertebrae while others have 7. In such cases, pre-operative radiography is advisable to positively identify the target operative site. Performing surgery at L5–L6 in such populations will result in the operative space adjacent to the lumbosacral space in some rabbits and at one space proximal in other cases. It is unknown if the biomechanical forces across the inter-lumbar joints are all equal or if there are differences between the joint adjacent to the lumbosacral joint and more proximal joints. Selecting L4–L5 as the operative site may minimize this potential problem, as L4–L5 is separated from the lumbosacral joint by at least one motion segment. Fusion masses at L4–L5 may be easier to harvest than a fusion mass at L5–L6 in cases where there are only 6 lumbar vertebrae. Finally, in many cases where there are only 6 lumbar vertebrae, the 6th transverse process may be quite narrow than the transverse process of L4 or L5. There may be no significance to this observation.

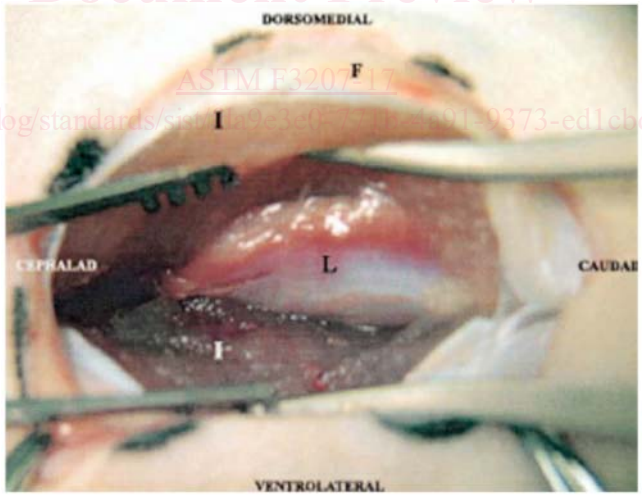
6.1.9.4 *Surgical Approach*—A representative description assuming an L5–L6 fusion site is described hereafter. A dorsal midline skin incision measuring approximately 6 cm in length is centered over the L5–L6 level. A full-thickness flap of skin and subcutaneous tissue is developed and retracted to one side. Approximately 2 cm lateral to the midline at the L5–L6 level, a 4-6 cm longitudinal incision is made through the lumbar fascia. Through this fascial incision, the iliocostalis muscle is divided exposing the underlying longissimus muscle (Fig. 2).

(1) To reach the transverse processes, blunt dissection is performed along the lateral border of the longissimus muscle. Exposure of the posterolateral fusion site is accomplished by elevating the iliocostalis muscle in a lateral direction off the transverse processes and intertransverse ligament. Dorsomedial retraction of the longissimus muscle is required to expose the medial aspect of the transverse processes and the pars interarticularis. Care should be taken to avoid inadvertent



NOTE 1—Dorsal view of the rabbit lumbosacral region. The iliac crests (IC) and the greater trochanters (GT). The intercrestal line (ICL), drawn from the most cranial aspect of one iliac crest to the other, will generally pass between the L6 and L7 spinous proceses. The interspinous distance at L6–L7 is substantially wider than at L5–L6 and L7–S1.

FIG. 1 Localization of the L5–L6 Fusion Site

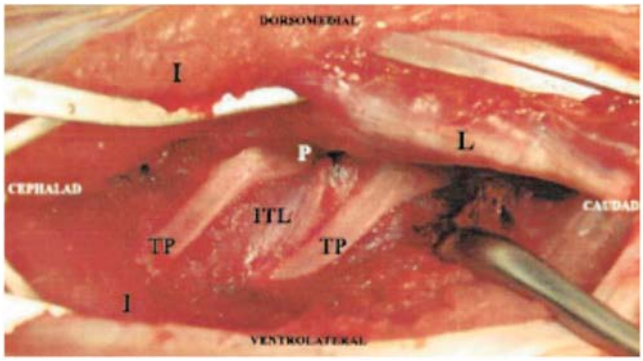


NOTE 1—After mobilizing the skin, the lumbar fascia (F) is vertically incised approximately 2 cm lateral to the spinous processes at the L5–L6 level. Through this fascial incision, the iliocostalis muscle (I) is divided exposing the underlying longissimus muscle (L).

FIG. 2 Surgical Approach: Superficial Dissection

exposure of adjacent facet joints or transverse processes at the proximal and distal levels. A small self-retaining retractor will maintain exposure of the two transverse processes and the intertransverse ligament (Fig. 3).

(2) To minimize bleeding during and after surgery, the dorsal branch of the segmental artery is cauterized as it passes with the posterior ramus through the operative field. As a means of limiting hemorrhage, it is also helpful to pack the wound with gauze upon completing the first surgical approach. After exposure and packing of the contralateral fusion site, retractors are replaced on the initial side to begin the decortication process. It is advisable to simultaneously palpate the left and right fusion sites to verify that the same level has been exposed on both sides of the spine.



NOTE 1—To reach the transverse processes, blunt dissection is performed along the lateral border of the longissi- mus muscle. Exposure of the posterolateral fusion site is accomplished by elevating the iliocostailis muscle (I) off the transverse processes (TP) and intertransverse ligament (ITL) and then retracted ventrolaterally. Dor- somedial retraction of the longissimus muscle (L) is required in order to expose the pars interarticularis (P).

FIG. 3 Surgical Approach: Deep Dissection Demonstration to Show the Anatomy

(3) Decortication of the transverse processes is performed with a motorized burr until punctate bleeding observed. Transverse process decortication should be performed as indicated by Fig. 4. The extent of decortication has been shown to be a determining factor in fusion rate, so care should be taken to ensure that decortication does not extend on to the vertebral body, as this may result in higher than expected fusion rates.

(4) The fifth lumbar root is vulnerable to injury as it exits the L5–L6 intervertebral foramen immediately dorsal to the plane of the intertransverse ligament and transverse processes. The lumbar plexus is also vulnerable to injury as its component nerves pass just ventral to the intertransverse ligament making it essential to preserve the integrity of this ligament during the exposure and decortication process.

6.1.9.5 *Harvest of Iliac Crest Bone*—Arthrodesis using autogenous bone from the ilium is often implemented as a control group in spinal fusion research using the NZW rabbit model. Working through the same dorsal skin incision, the cranial and lateral surface of the iliac crest is exposed in a subperiosteal plane (Fig. 5, top). This central part of the iliac wing contains the greatest amount of cancellous bone and can be localized by palpation of the medial iliac spine (Fig. 5, bottom). The recommended quantity of graft, 2.5 to 3.0 cc per side of the spine, generally requires harvesting a significant proportion of both ilia. During graft harvest, it is critical to be gentle when elevating the muscles off the inner cortex when taking the tricortical ilac crest graft. Dissection in this area can traumatize the neurovascular structures that pass through the sciatic notch leading to serious hemorrhage and/or sciatic nerve palsy. Some amount of palsy (~10%) is an expected consequence of harvesting the recommended 2.5 to 3.0 cc of graft.

6.1.9.6 Morselize the corticocancellous autograft bone with a rongeur into <5 mm irregular pieces. Make sure to remove all soft tissues from the morselized iliac crest bone.

6.1.9.7 Decortication and grafting material should be confined to the medial one half of the two transverse processes (i.e., half of the transverse process that is close to the vertebral body). As shown in Fig. 4, the graft material should be placed on top of the red zones and filled in between the two transverse processes. Place either the iliac bone autograft or the test article between the transverse processes in the paraspinal bed, paying particular attention to placing the graft material along the medial half to one-third of the transverse processes where decortication was done (Fig. 6).

6.1.9.8 Close the fascial incisions with 3-0 absorbable suture and the skin edges are approximated using absorbable 3-0 or 4-0 suture, with or without 35W staples.

6.2 *Recovery—Post-operative Care and Analgesics:*

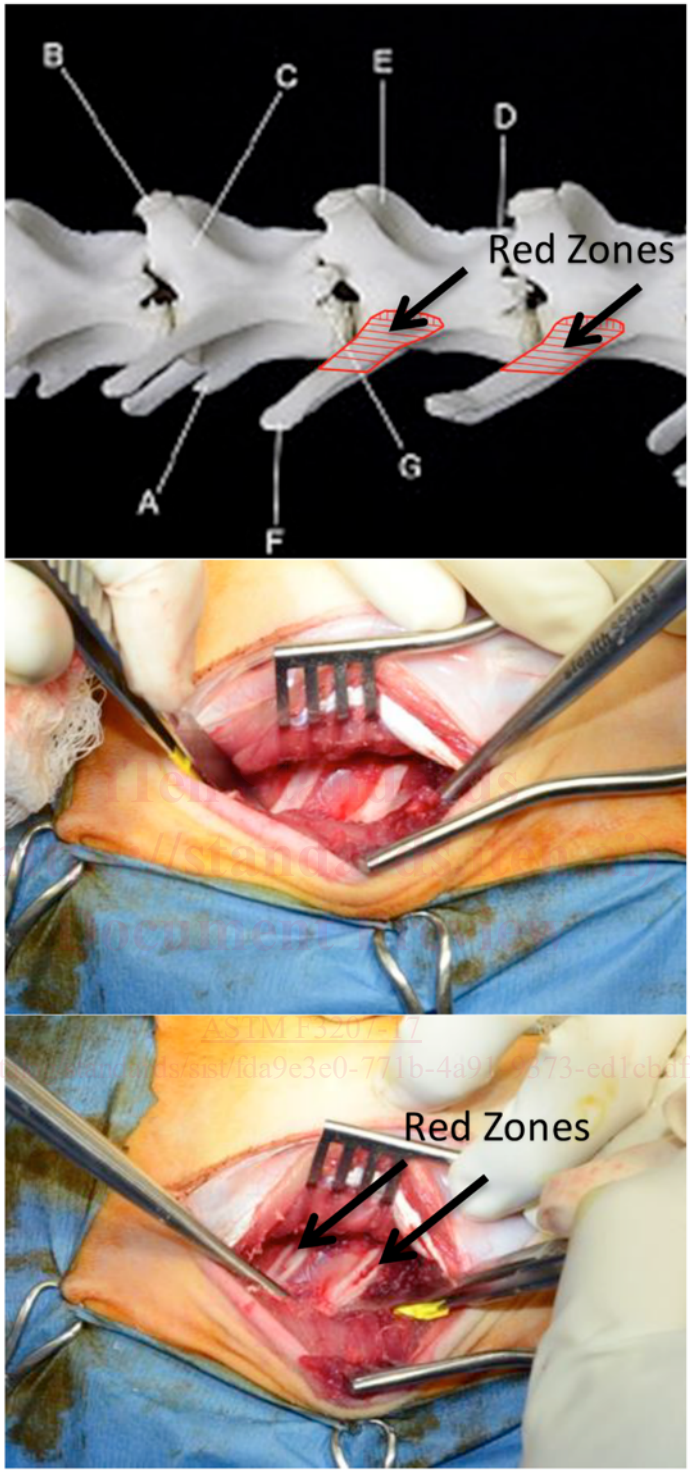
6.2.1 Warm blankets and heated mats should be used both intra-operatively and post operatively to keep the animal's body temperature within normal range. For analgesics, 0.05 mg/ kg buprenorphine should be administered subcutaneously approximately 6 hours after the first dose. The second dose of buprenorphine should give the animals an adequate plane of analgesia until the fentanyl patch reaches therapeutic levels. Fentanyl patches should be replaced approximately every 72 hours until the animal is no longer deemed painful. Pain levels can be monitored based on how well the animals are eating, posture and ease of movement within the cage. When these 3 observations are deemed normal, the animal can then be considered pain free. If not all of those items are normal, then consideration needs to be given for additional analgesics.

6.2.2 During the first hour after surgery, pulse and respiratory rate are monitored; supplemental fluids are administered intravenously or subcutaneously as needed. Animals should be monitored until ambulatory, handled carefully, and then returned to their cage.

6.3 *Post-operative Care:*

6.3.1 The general condition of the rabbit should be monitored twice each day for the first 3 days after surgery, followed by once a day for the remainder of the study. If used, skin staples are removed 2 weeks after the operation.

6.3.2 Post-operative anorexia in rabbits may be a serious complication and can result in death within 4-6 days. To that end, rabbits should be encouraged to eat after surgery. Rabbits may be supplemented with fresh fruits or vegetables (apples, carrots, timothy hay cubes) during the acute post-operative period if dietary intake of their normal ration is reduced. A particularly effective supplement is Critical Care by Oxbow Animal Health, which is a highly palatable, high fiber supplement for herbivores that is highly effective in stimulating



NOTE 1—(Top) Schematic representation of the decortication area. (Middle) Virgin transverse processes. (Bottom) Decorticated Transverse processes.

FIG. 4 Decortication of Transverse Processes

appetite in post-operative rabbits. Rabbits are weaned off supplements as they regain their appetite for their normal ration.

6.4 *Recommended Observations:*

6.4.1 *General Health*—Observations can occur through close, cage-side observations. If any abnormal clinical signs including signs of inflammation and/or infection, hind limb