4 – Special Control Summary

Butler, Jennie C

From: Lisa Simpson [lsimpson@rtix.com]
Sent: Wednesday, August 16, 2006 5:53 PM
To: Hanna, Myrna
Cc: Butler, Jennie C
Subject: RTI Bone Heterograft Reclassification Petition

Dear Myrna,

I wanted to let you know that none of the information submitted in the "Reclassification Petition, Bone Heterograft", dated August 10th 2006 is considered to be confidential by RTI. As such, I am giving FDA permission to distribute the information as necessary in support of the reclassification efforts.

Thank you,
Lisa

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Special Control Summary for a Bone Heterograft Vertebral Body Replacement Device

The following summary describes how FDA special controls were applied to the Sterling Impacted Cortical Ring, a bone heterograft intervertebral body replacement device.

1. Infection

Infection of the soft tissue, bony tissue, and the disc space is a potential risk to health associated with all surgical procedures and implanted spinal devices. Material composition or impurities, wear debris, operative time, and operative environment may compromise the vascular supply to the area or affect the immune system, which could increase the risk of infection. Improper sterilization or packaging may also increase the risk of infection.

FDA recommends sponsors provide sterilization information for the finished VBR device in accordance with the "Updated 510(k) Sterility Review Guidance K90-1; Final Guidance for Industry and FDA".

The BioCleanse® Tissue Sterilization Process is used to sterilize the bovine bone prior to packaging. The STERLING® Impacted Cortical Ring is packaged in inner foil pouch, and sealed in a poly/foil outer pouch. Terminal sterilization is performed using gamma irradiation at a minimum dose of 25kGy. The sterilization process used for the STERLING® Impacted Cortical Ring is the same as that used for the STERLING® Interference Screw HT (K060253, K052405). The sterilization validation study conforming to the requirements of ISO 11137-1 and follows the recommendations of FDA Guidance Document K90-1:

Compliance with Guidance Document *"Updated 510(k) Sterility Review Guidance K90-1; Final Guidance for Industry and FDA"* (issued 11/16/2001)

Gamma irradiation is considered a traditional method of sterilization according to this guidance document.

- *Sterilization method that will be used*
  Gamma irradiation

- *Description of the method that will be used to validate the sterilization cycle, but not the validation data itself*
  The AAMI TIR 27 method that was used to substantiate a 25kGy sterilization dose is based on the standard distribution of resistances (SDR) found in Method 1 of ANSI /AAMI / ISO 11137:1994, Sterilization of health care products – requirements for validation and routine control – Radiation Sterilization (ISO 11137). Method 1 is a procedure for establishing a sterilization dose based on determination of the product bioburden and comparison of that bioburden information to the SDR model population having a defined resistance to radiation. The AAMI TIR 27 method incorporates experimental verification showing that the response of the product bioburden is less resistant that that of the SDR, substantiating 25kGy as an appropriate 10^{-6} Sterility Assurance Level (SAL) dose.

The validation study was performed by selecting a Master Product from the items that are included in the STERLING® Product Family. A bioburden estimate was then established for the Master Product. A 10^{-1} SAL verification dose was established for the Master Product based on the bioburden estimate. The test of sterility was conducted on 10 product samples exposed to the verification dose. Statistical verification was confirmed in the verification experiment, substantiating the radiation dose of 25kGy to achieve the desired SAL of 10^{-6} for the established bioburden estimate of the representative product.

- *Description of the packaging to maintain the device's sterility, not including package integrity testing data*
  The STERLING® Impacted Cortical Ring is packaged in a foil-on-foil primary pouch, which is then sealed in a foil-to-poly outer pouch.

- *If sterilization involves EtO, the maximum levels of residues of EtO and ethylene chlorhydrin which remain on the device (note: ethylene glycol residue level was dropped from this updated guidance because the recognized standard, ISO 10993-7, does not include measurement of ethylene glycol residues).*
  Sterilization does not involve EtO.

- *If the product is labeled "pyrogen free," a description of the method used to make the determination, e.g., limulus amebocyte lysate (LAL)*
  The STERLING® Impacted Cortical Ring will have an endotoxin limit of 0.5EU/ml, determined either by testing each lot with LAL or by process validation in accordance with AAMI ST72:2002.

- *The sterility assurance level specification (SAL) (e.g., 10^{-6} for all devices, except 10^{-3} for devices only contacting intact skin)*
  SAL = 10^{-6}

- *In the case of radiation sterilization, the radiation dose*
  Dose = 25kGy to 32kGy.

2. BSE transmission

With the use of bovine materials for VBR devices, there is a risk that Bovine Spongiform Encephalopathy (BSE) may be present in source cattle and may be transmitted to the patient through implantation of the device. However, according to a World Health Organization (WHO) scientific consultation, bovine bone is a Category IV material with no detectable infectivity for BSE.[1] Despite the low risk of transmitting BSE to patients through the use of bovine bone, RTI has implemented special controls to further reduce this risk.

In cattle naturally infected with BSE (i.e., commercially reared animals not part of a specially designed experiment), the BSE agent has been found only in brain tissue, in the spinal cord, and in the retina of the eye. In specially designed experiments, the BSE agent has been detected in the brain, spinal cord, distal ileum, dorsal root ganglia (DRG), trigeminal ganglia, and, possibly, the bone marrow of deliberately infected cattle from whose tissues were collected and analyzed for the BSE agent. Some tissues, such as brain and spinal cord, contain higher levels of BSE infectivity than others. The BSE agent has never been detected in the muscle tissue of BSE-infected cattle, regardless of the age of the animal.[2] RTI does not use materials that are derived from any of the following specified risk materials: brain, skull, eyes, trigeminal ganglia, spinal cord, vertebral column (excluding the vertebrae of the tail, the transverse processes of the thoracic and lumbar vertebrae, and the wings of the sacrum), and dorsal root ganglia (DRG) of cattle 30 months of age and older, and the tonsils and distal ileum of the small intestine of all cattle.[3]

The WHO guideline "WHO Infection Control Guidelines for Transmissible Spongiform Encephalopathies" identifies the USA as a Category II ("unlikely but not excluded") country with regards to its geographic BSE risk assessment.[4] The STERLING® Impacted Cortical Ring is composed of bone derived from a closed herd to further minimize the risk of BSE contamination. All RTI STERLING bovine xenograft products are made from materials obtained from a bovine closed herd on Prather Ranch. The herd has been closed since 1992. The cattle are bred either via artificial insemination or using ranch raised closed herd bulls. Detailed records are kept on each animal, which include information such as maternal lineage, animal birth date, vaccination and health information. Records of exposure to infectious agents, documentation concerning the various breeds and lineage as well as any incident that may have affected the herd and vaccinations are kept permanently at the Prather Ranch facility. In addition, an electronic copy of this information is available on a computer database, CattlePro 2000 Prime. This database is continually updated and is available to RTI for review prior to release of

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[1] "Report of a WHO Consultation on Medicinal and other Products in Relation to Human and Animal Transmissible Spongiform Encephalopathies", page 15, WHO/EMC/ZOO/97.3, www.who.int/entity/biologicals/publications/en/BTSE97mar24.pdf

[2] FSIS "Current Thinking On Measures That Could Be Implemented To Minimize Human Exposure To Materials That Could Potentially Contain the Bovine Spongiform Encephalopathy Agent", January 15, 2002, http://www.fsis.usda.gov/oa/topics/bse_thinking.htm

[3] USDA Interim Final Rule, "Prohibition of the Use of Specified Risk Materials for Human Food and Requirements for the Disposition of Non-Ambulatory Disabled Cattle", Docket 03-025IF, http://www.fsis.usda.gov/OPPDE/rdad/FRPubs/03-025IF.pdf

[4] WHO Surveillance and control, http://www.who.int/zoonoses/diseases/surveillance/en/, downloaded April 22, 2006.

the tissue for processing. All members of the closed herd are examined annually by a veterinarian. Comprehensive veterinary records are maintained by a single veterinary clinic that has cared for the herd since 1964. No growth hormones are administered to the cattle.

Prather Ranch consists of a number of ranches located in high mountain valleys in California. The ranches are isolated properties in clean environments. Severe temperature variations reduce the growth of animal pathogens, and regular soil and water testing ensure a pure environment. In order to minimize contact with other cattle, the closed herd cattle graze on an area of the ranch which contains large freshwater springs. The slaughterhouse facility was constructed in 1995 to meet the specifications of the pharmaceutical industry, USDA, FDA and ISO 9001. Only closed herd cattle are slaughtered in this facility. All facilities and equipment are sanitized prior to use, and all processing is performed under sanitary conditions.

There is only one slaughterhouse, at Prather Ranch that is and will be used for the slaughter of the closed herd animals. Only animals from the Prather Ranch closed herd are slaughtered there. 100% of the slaughters are inspected by a certified USDA representative, during which time a Daily Disposition Record (FSIS 6200-14) is filled out. This form serves as a log for noting disease, unsanitary conditions, injuries, metabolic disorders, etc. for the cattle. Any significant findings are listed on the Certificate of Ante-mortem or Postmortem Disposition of Tagged Animals (FSIS 6000-13). A certified veterinarian licensed by the USDA must perform a follow up of any finding listed on form FSIS 6000-13.

Answers to points raised by the FDA Guidance Document "Medical Devices Containing Materials Derived from Animal Sources (Except for In Vitro Diagnostic Devices)" (Issued 11/6/98)

1. *All materials in a device which are derived from a bovine source should be identified. Examples are: bovine pericardium used in heart valves, bovine viscera used in gut sutures, bovine bone used in dental implants, and bovine collagen used in lacrimal plugs. These also include devices which are exposed to materials of bovine origin during manufacture (e.g., human cells grown in media containing fetal calf serum, tissue culture cells exposed to bovine trypsin.)*

   The STERLING® Impacted Cortical Ring is composed entirely of bovine bone. Bovine bone has been given a Category IV (no detectable infectivity) rating by WHO[5]. The skull and vertebrae are never used for the Sterling products and the head and spine are maintained intact during slaughter and harvest of bones used by RTI.

2. *The bovine material should come from cattle which have not originated from or resided in a country where BSE has been diagnosed or which presents a significant risk of introducing BSE. This list of countries is maintained by the USDA and codified in 9 CFR 94.18. The countries currently identified include all countries of Europe.*

[5] "Report of a WHO Consultation on Medicinal and other Products in Relation to Human and Animal Transmissible Spongiform Encephalopathies", WHO/EMC/ZOO/97.3, www.who.int/entity/biologicals/publications/en/BTSE97mar24.pdf

The bovine bone used to manufacture the STERLING® Impacted Cortical Ring is derived from a closed herd maintained by Prather Ranch in Macdoel, California.

3. *Traceable records should be maintained by the device manufacturer for each lot of bovine material and each lot of FDA-regulated product. Records should indicate the country of origin and residence of the animals. The bovine tissue source (e.g. bone, heart valve, ligament/tendon) should also be indicated.*
   Traceable records are maintained for each lot of bone. Records indicate the country of origin, as well as the specific ranch. Only bone is used as a source material.

4. *If the manufacturer certifies that the bovine-derived material is only available from a country where BSE is known to exist, then the manufacturer should provide evidence to indicate that the BSE agent is inactivated during the manufacturing process. A detailed description of the manufacturing process should be submitted.*
   This section is not applicable because the source of our material is from a country where BSE is not known to exist.

5. *The FDA has recently changed its position with regard to the use of gelatin. A guidance document has been issued regarding the use of gelatin in FDA-regulated products for human use.*
   This point is not applicable because the STERLING® Impacted Cortical Ring is not composed of gelatin.

Additional Applicable Standards and Certifications

For the purpose of distribution of Sterling products in the European Union, the source herd, slaughter and processing methods have been audited by RTI and an independent third party to verify that they meet the requirements of EN ISO 12442-1, -2 and -3. EN ISO 12442-1:2000[6] provides additional requirements and guidance beyond for the evaluation of risks associated with medical devices utilizing animal tissues or derivatives. EN ISO 12442-2:2000[7] specifies requirements for controls on the sourcing, collection and handling (which includes storage and transport) of animals and tissues for the manufacture of medical devices utilizing materials of animal origin. EN ISO 12442-3:2000[8] specifies requirements for the validation of elimination and/or inactivation of viruses and/or TSEs during the manufacture of medical devices utilizing materials of animal origin.

For the purpose of exporting Sterling products, RTI obtains certification of its products from the USDA. For example, the Sterling products are certified with the following statements: "The Sterling *name of device* is produced under a system designed to ensure that it is free from (unlikely to transmit) disease agents, including transmissible spongiform encephalopathies (TSEs). This system includes the following measures. The materials are derived exclusively from animals that were born and raised on the Prather Ranch in California. Materials are derived from animals whose dams were all born and raised on the same Prather Ranch. No maternal animals have been introduced into the

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[6] EN ISO 12442-1:2000, Animal tissues and their derivatives utilized in the manufacture of medical devices – Part 1: Analysis and management of risk.
[7] EN ISO 12442-2:2000, Animal tissues and their derivatives utilized in the manufacture of medical devices – Part 2: Controls on sourcing, collection and handling.
[8] EN ISO 12442-3:2000, Animal tissues and their derivatives utilized in the manufacture of medical devices – Part 3: Validation of the elimination and/or inactivation of viruses and transmissible agents.

Prather Herd since 1975. No male animals have been introduced into the Prather Herd since 1992. No case of bovine spongiform encephalopathy has ever been reported on the Prather Ranch. Source animals are grass/grain fed and have never been fed meat-and-bone meal or greaves. The materials are not derived from any of the following specified risk materials: the brain, skull, eyes, trigeminal ganglia, spinal cord, vertebral column (excluding the vertebrae of the tail, the transverse processes of the thoracic and lumbar vertebrae, and the wings of the sacrum), and dorsal root ganglia (DRG), tonsils, and the distal ileum of the small intestine of ruminants."

Bovine bone from Prather Ranch is used to manufacture Class II FDA-cleared medical devices. This includes the Sterling Interference Screw HT (K060253, K052405) and Sterling Cancellous Chips and Sterling Cancellous Cubes (K051615). Of these devices, the Sterling Cancellous Chips and Sterling Cancellous Cubes have been cleared for use in the spine. A comprehensive search of the medical literature[9] from 1966 to the present did not yield any evidence for increased risk of BSE transmission when transplanting bovine bone in the spine versus transplanting bovine bone in other anatomical regions.

[9] PubMed, http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?DB=pubmed

3. Adverse tissue reaction

Adverse tissue reaction is a potential risk to health associated with all implanted devices. The implantation of the device will elicit a mild inflammatory reaction typical of a normal foreign body response. Incompatible materials or impurities in the materials and wear debris may increase the severity of a local tissue reaction or cause a systemic tissue reaction. If the materials used in the manufacture of the device are not biocompatible, the patient could have an adverse tissue reaction.

Biocompatibility testing was performed on STERLING® bone in accordance with FDA Good Guidance Practice Document G95-1 (ISO 10993) to alleviate any concerns regarding the biocompatibility of the current device. An immunotoxicity test was also performed in consultation with FDA. In addition, animal testing was performed according to ASTM F981 using bovine bone processed in the same manner as the current device. Finally, an implant study in a sheep model was undertaken to compare the antigenicity of the bovine bone used for the current device to controls. The results of these studies indicate that the bovine bone of the Sterling Impacted Cortical Ring is biocompatible.

<u>FDA Good Guidance Practice Document G95-1 (ISO 10993) Testing</u>

The following tests recommended in Table 1 of the G95-1 guidance document were performed in compliance with ISO 10993:

- Cytotoxicity
- Sensitization
- Genotoxicity
- Implantation

The cytotoxicity results indicated toxicity to mouse fibroblast cells. The toxicity of these materials to mouse fibroblasts, however, does not reflect the biocompatibility of the STERLING® material in the *in vivo* setting. Histology from an implant study in sheep showed good incorporation of the implant into the host (please see the sheep study summary below). This shows that any potential cytotoxic effects of the material do not inhibit bone growth. We chose to also perform the other tests of Table 1 of the G95-1 guidance document, which are all *in vivo* tests:

- Irritation or intracutaneous reactivity
- Acute system toxicity
- Sub-chronic (sub-acute) toxicity

Table 1: G95-1 Biocompatibility Testing Results

Test Name	Result
MEM elution Cytotoxicity	Toxic
Sensitization	Grade I, Weak sensitizer
Intracutaneous Reactivity	Non-irritating
System Toxicity (Acute)	Non-toxic
Sub-chronic Toxicity (Sub-Acute)	Non-toxic
Genotoxicity	Non-mutagenic
Implantation	Meets requirements of test

All of the tests were performed according to ISO-10993 standards. After repeating the cytotoxicity test and receiving a second toxic result, an independent assessment of all the test data was sought.

The independent reviewer determined that "due to the results of the *in vivo* testing performed that the product meets *in vivo* biocompatibility requirements and may be considered safe for clinical use under appropriate guidance documents." The cytotoxicity results raised the concern that the materials of this device may inhibit bone growth or remodeling and interfere with its *in vivo* performance. Further biocompatibility testing was performed *in vivo,* first in a rat model and then in a sheep model.

In vitro Immunotoxicity – RAW Cell Production of TNF-α

The induction of TNF-α was tested using RAW 264.7 cells for the STERLING® Interference Screw ST, which is composed of the same material processed in the same manner as the STERLING® Impacted Cortical Ring. The STERLING® Interference Screw ST and STERLING® Impacted Cortical Ring are manufactured by machining bovine bone into the finished shape, subjecting the device to the BioCleanse® Tissue Sterilization Process, packaging and terminally sterilizing the device using gamma irradiation. As a control, bovine interference screws were also manufactured, packaged, and terminal sterilized, but without the use of the BioCleanse® Tissue Sterilization Process.

Three forms of the interference screws were tested: (1) intact, (2) crushed into crude bone powder using a mortar and pestle, and (3) ground into a powder using a cryogenic mill. The different physical conditions were tested in order to verify that there were no elements within the interior matrix that would elicit the production of TNF-α when exposed as the implant remodels *in vivo*.

The overall result was that the samples of (1) whole, (2) crude bone powder, and (3) fine bone powder STERLING® Interference Screw ST, subjected to the BioCleanse® Tissue Sterilization Process, exhibited a lower TNF-α induction score than the non-BioCleansed samples. None of the BioCleanse® processed interference screws exhibited a TNF-α induction score above the LPS control, which represents the 0.5 EU/mL maximum for medical devices. STERLING® bone has a negligible immunogenic response.

In vivo Biocompatibility – ASTM F-981 Rat Model

Biocompatibility testing was also performed by RTI according to ASTM F981 in order to determine the biocompatibility of bovine implant materials by measuring immune and other biocompatibility responses. Negative controls and bovine implants were implanted in Sprague-Dawley rats. The implants were excised after 28 days and sent off for histologic evaluation by a board certified licensed pathologist. It was his conclusion that while a slight foreign body response was observed, it would not likely interfere with osteogenesis and bone repair. The tissue response was mild and would not interfere with bone formation. See the photomicrographs in Figure 1 for a histological example of a negative control and a bovine implant. Note that the fibrous tissue is more dense and cellular in the bovine implant than the negative control, but the inflammation is minimal.

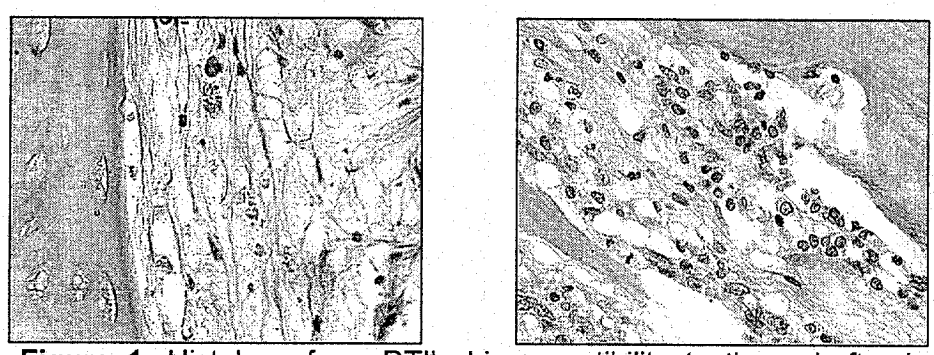

Figure 1: Histology from RTI's biocompatibility testing. Left: photomicrograph of negative control; Right: photomicrograph of bovine implant (H&E, 400x)

To further clarify the effects of the processing and materials on remodeling of the bone, another animal implant study was performed. Bovine bone processed in the same manner as the current device was implanted in sheep, and compared with bovine bone processed using traditional aseptic methods, bovine bone processed with the BioCleanse® Tissue Sterilization Process but not terminally irradiated, and sheep allograft processed using traditional aseptic methods.

In vivo Biocompatibility – Sheep Study

The ability of STERLING® Impacted Cortical Ring material to remodel *in vivo* was evaluated in a sheep model. Twenty-four skeletally mature sheep each underwent staged bilateral surgical procedures. Two prepared 5 x 25mm tunnels in the medial proximal tibia received the test article bone dowels. Implant sites designated for histopathological evaluation received full-length implant bone dowels (5 x 24mm). Implant sites designated for biomechanical evaluation received short implant bone dowels (5 x 5mm) and a Delrin dowel (5 x 20mm) to fill and mark the tunnel for relocation at time of sacrifice. Six sheep were implanted with untreated aseptic bovine xenograft in one limb and twelve weeks later received untreated aseptic bovine xenograft in the contra lateral limb (Group 1). A second group of six sheep were implanted with BioCleanse®-treated bovine xenografts in the same manner as the first group (Group 2). A third group of six sheep received untreated but aseptically handled sheep bone allografts (Group 3). A fourth group of six sheep received BioCleanse®-treated bovine xenograft sterilized with gamma irradiation in the same manner as the previous groups (Group 4). Group 4 samples were made using the same processing

and sterilization methods as are used to manufacture the STERLING® Impacted Cortical Ring. All of the animals were sacrificed 24 weeks following the initial surgery. Half of the implants and native bone at an adjacent site were compression tested using an Instron 4302 and the other half of the implants were histopathologically evaluated.

The histopathological results at both 12 weeks and 24 weeks indicate that the BioCleanse®-treated bovine bone remodels comparably to sheep allograft bone. The bones were embedded in plastic and ground sections were stained with Giemsa. The slides were examined with light microscopy for morphologic analysis. In each group at each time period, an average for each parameter was determined. At 12 weeks, the most active response was in Group 1 (untreated bovine xenograft) where the implant was being actively removed by large numbers of osteoclasts accompanied by mixed inflammatory cells and fibrosis:

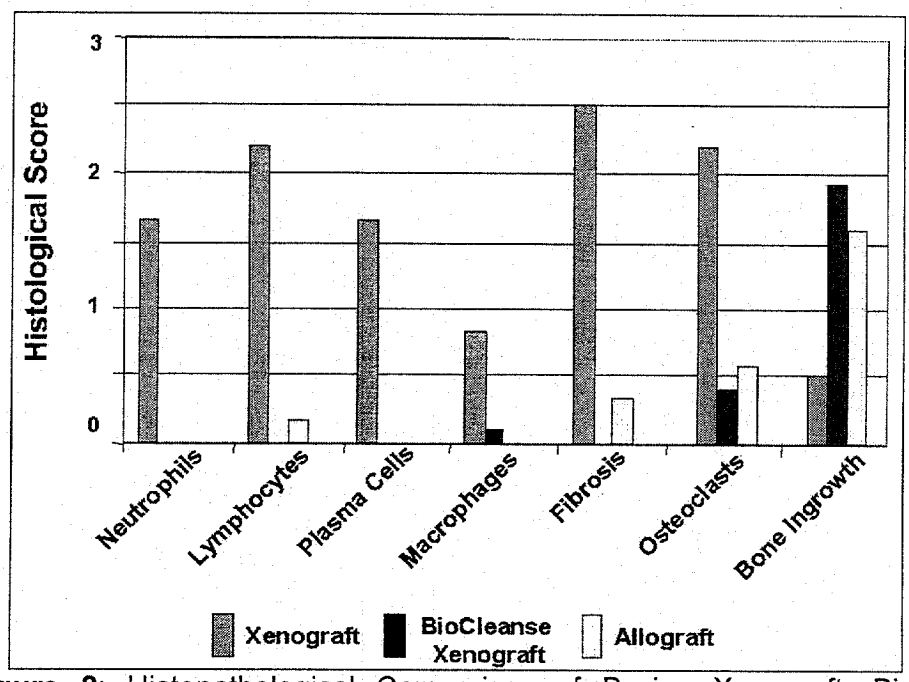

Figure 2: Histopathological Comparison of Bovine Xenograft, BioCleansed Bovine Xenograft, and Sheep Allograft

However, at this 12-week time point, bone ingrowth was greatest in Group 2 and next greatest in Group 3 followed by Group 4 and the least in Group 1. The greatest overall reactivity was in Group 1 tissues. Examples of histology slides for Group 1 and Group 2 at twelve weeks can be found in Figure 3.

Both gamma irradiated and non-irradiated BioCleanse®-treated bovine bone elicited healing responses similar to that of fresh frozen allograft when implanted into the proximal tibia of sheep.

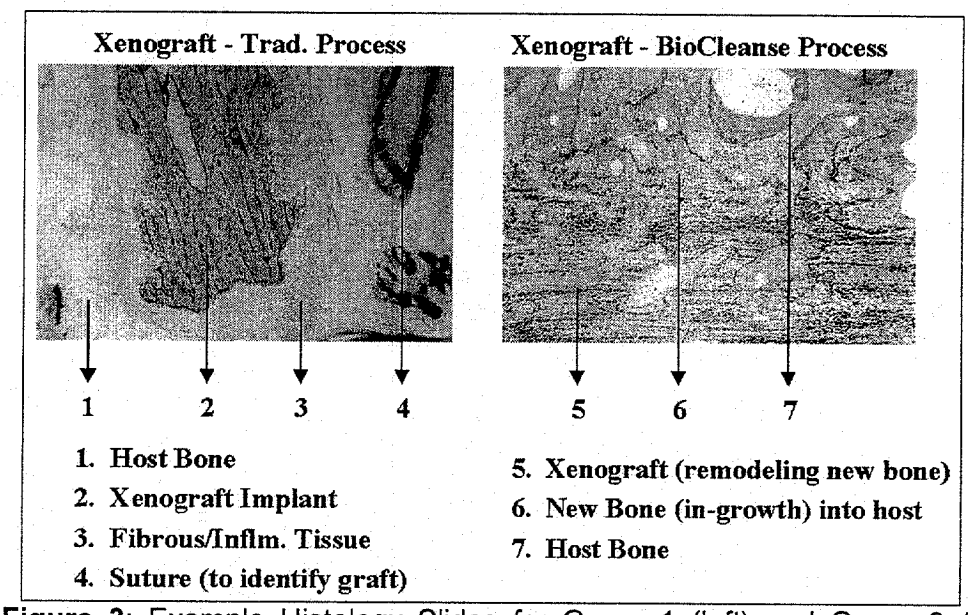

Figure 3: Example Histology Slides for Group 1 (left) and Group 2 (right) at Twelve Weeks

Both of the BioCleanse®-treated groups were found to be similar pathologically and biomechanically to the allograft control. The data were analyzed for stress at maximum load, strain at maximum load, and stiffness at maximum load. Stress at maximum load was determined by averaging the group data for both implant bone and native bone and dividing the maximum load (N) by the area (19.63mm^2). Figure 4 shows the results of the stress analysis, and Figure 5 shows the results of the stiffness analysis.

The results of this study indicate that the BioCleanse® Tissue Sterilization Process renders the bovine xenograft essentially equivalent to allograft control. Irradiation of the BioCleanse® treated xenograft to a small degree increases the biological reactivity of the implant, but has little influence on the biomechanical properties of healing of the implant.

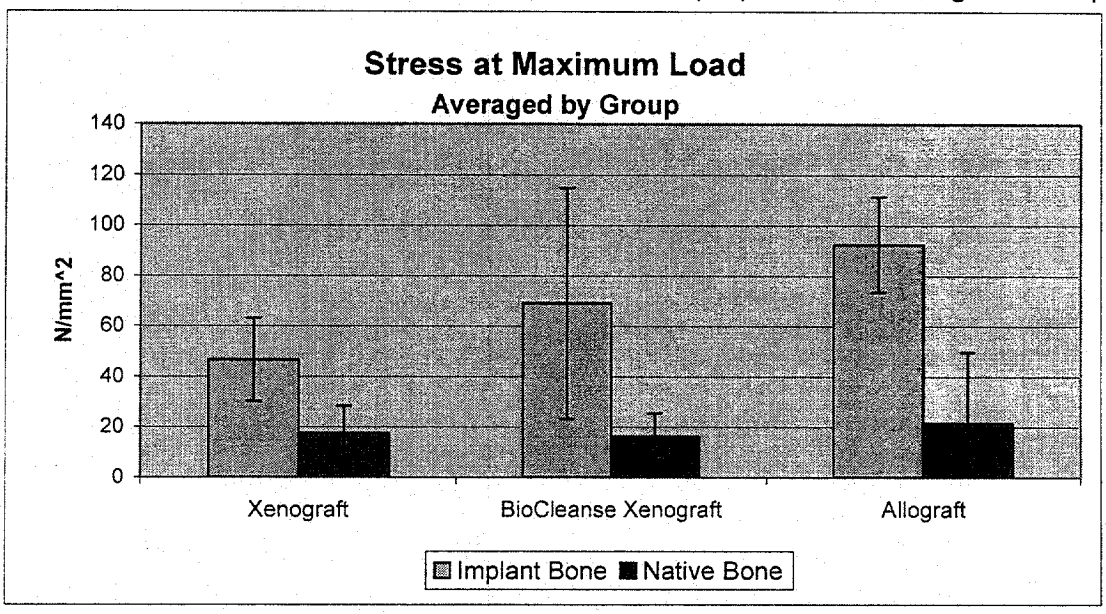

Figure 4: Stress at Maximum Load at Twelve Weeks

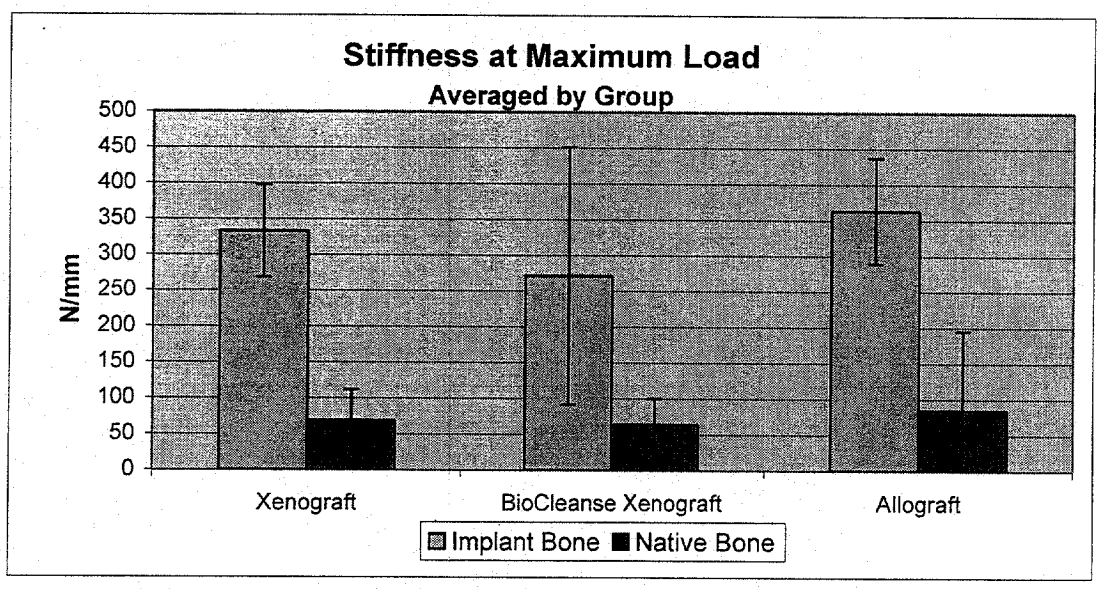

Figure 5: Stiffness at Maximum Load at Twelve Weeks

A study was added as an addendum to this sheep study, with the objective of assessing the antigenicity of traditionally processed allograft bone in comparison to bovine bone processed by the BioCleanse® Tissue Sterilization Process. Specifically, this study assessed the ability of bovine bone treated by aseptic processing (Trad), the BioCleanse® Tissue Sterilization Process (Bc), and the BioCleanse® Tissue Sterilization Process followed by gamma irradiation (BcG) to elicit an antigenic response when implanted in a discordant recipient (sheep). Minor changes were made in executing the addendum due to difficulties in timely receipt of reagents, and access to end point measuring equipment. Changes involved only a reduction in the number of time points for assessing the memory response of recipients, and did not alter the conclusions of the study.

The following graph summarizes the memory response of the various groups when challenged with the implant material at time points indicated in the X-axis. The legend for the graph depicts respective groups of recipients and the implant material used to measure the recall response. The data are presented as the average stimulation index (fold increase in response to challenge material over media control) for each group of recipients with their respective standard errors of mean. As seen below, recipients receiving traditionally processed bovine bone (Trad) demonstrated a significant response ($p = 0.008$, t-test) at 1 month post-surgery to the implant material (Trad) in comparison to their pre-bleed levels. This response tapered to pre-bleed/background levels at 3 months, and continued at 6 months (data not shown). Amongst the other groups, the BioCleanse®-implant recipient group (Bc) tended to show a similar response at 1 month post-implant, but did not achieve statistical significance ($p > 0.05$). The allograft (Sheep), and gamma-irradiated BioCleanse® (BcG) implant recipients failed to demonstrate any measurable recall response to their respective implants.

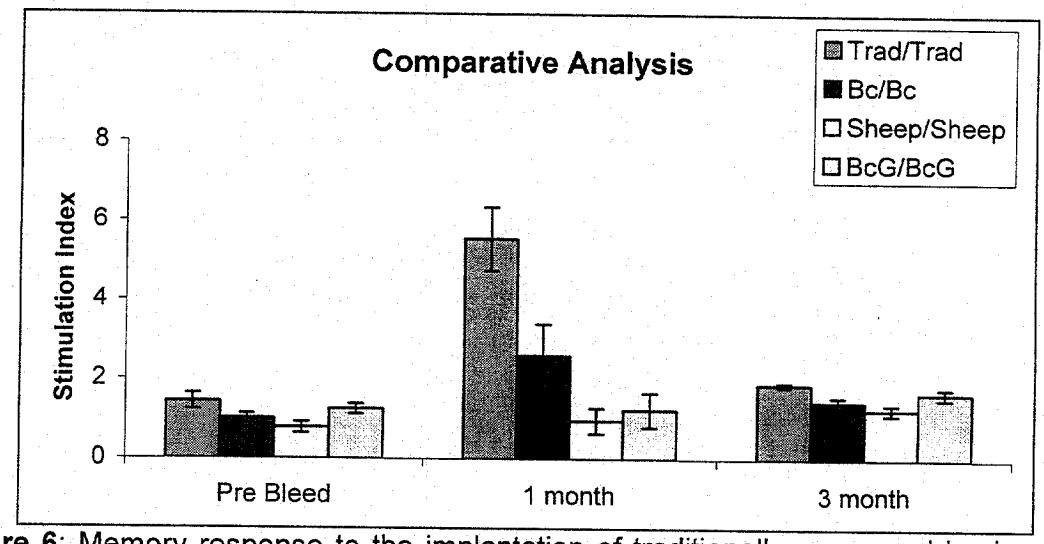

Figure 6: Memory response to the implantation of traditionally processed bovine bone (Trad), BioCleanse®-processed bovine bone (Bc), traditionally processed sheep bone (Sheep), and bovine bone processed by BioCleanse® followed by gamma irradiation (BcG).

The following series of graphs detail the recall response of each group of recipients (mentioned in the titles of each graph) when challenged with all four types of implant materials (detailed in legends for each graph). As seen earlier, data are presented as stimulation index (Y-axis), and the time points when assays were performed indicated in the X-axis. Sheep receiving the traditionally processed bovine bone implants (Trad) demonstrated the highest recall response at 1-month post surgery, with significant responses ($p<0.05$, t-test) only to the implants they received when compared to pre-bleed levels. A similar pattern was also noticed in animals receiving BioCleanse® implants, without statistical significance to pre-bleed levels. Responses from all 4 groups of implant recipients reached background/pre-bleed levels by 3 months post-implant, as mentioned earlier.

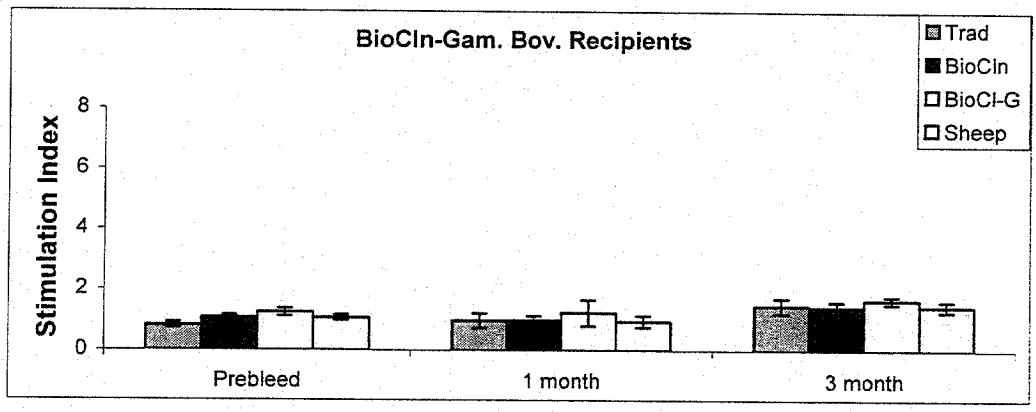

Figure 7: Response of sheep, post-implantation of bovine bone subjected to BioCleanse® and gamma irradiation, to traditionally processed bovine bone (Trad), BioCleanse®-processed bovine bone (BioCln), BioCleanse®-processed and terminally irradiated bovine bone (BioCl-G), and traditionally processed sheep allograft (Sheep).

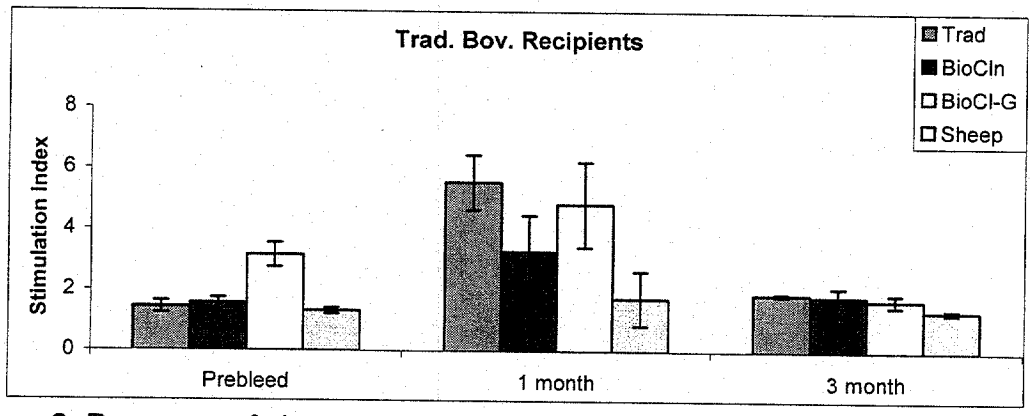

Figure 8: Response of sheep, post-implantation of traditionally-processed bovine bone, to traditionally processed bovine bone (Trad), BioCleanse®-processed bovine bone (BioCln), BioCleanse®-processed and terminally irradiated bovine bone (BioCl-G), and traditionally processed sheep allograft (Sheep).

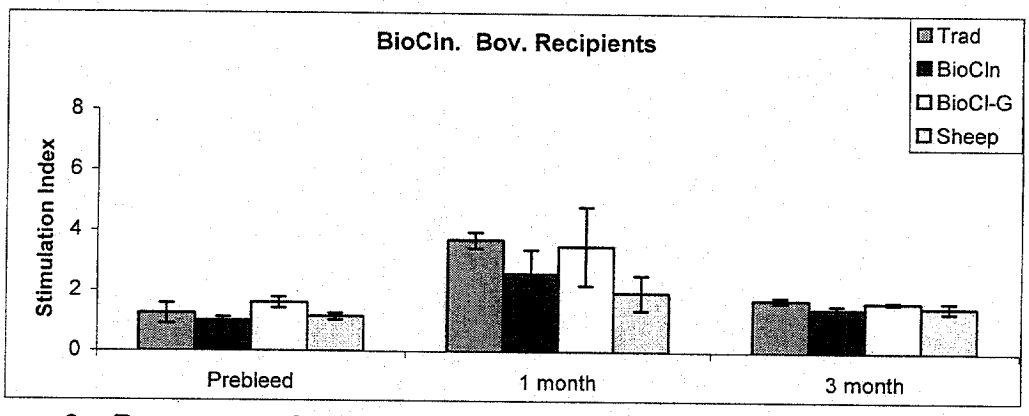

Figure 9: Response of sheep, post-implantation of bovine bone subjected to BioCleanse®, to traditionally processed bovine bone (Trad), BioCleanse®-processed bovine bone (BioCln), BioCleanse®-processed and terminally irradiated bovine bone (BioCl-G), and traditionally processed sheep allograft (Sheep).

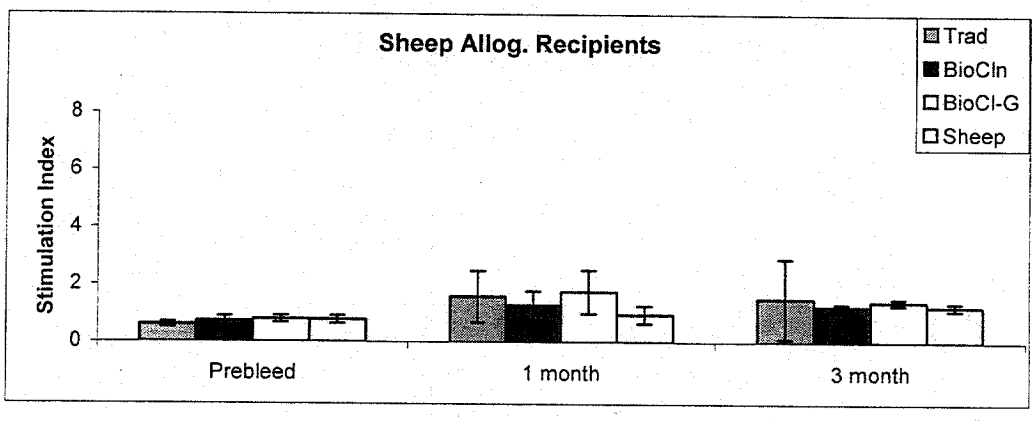

Figure 10: Response of sheep, post-implantation of traditionally-processed sheep allograft, to traditionally processed bovine bone (Trad), BioCleanse®-processed bovine

bone (BioCln), BioCleanse®-processed and terminally irradiated bovine bone (BioCl-G), and traditionally processed sheep allograft (Sheep).

Results from this study clearly point to the presence of antigenic components in minimally processed (traditional) bone grafts. These components are present at levels that are capable of evoking a recall or memory response when peripheral blood mononuclear cells from the recipients were tested. The study also indicated that the BioCleanse® Tissue Sterilization Process was able to significantly reduce these bone associated antigenic elements to levels incapable of eliciting a significant recall response. The recall responses measured in these studies appear to be of a transient nature as levels declined to pre-bleed or background levels by 3 months.

These results suggest that the antigenic elements associated with bone grafts (as limited by this model) can influence the early rather than later phases of remodeling. This hypothesis is strongly supported by the quantitative histopathology report from the study. For example, evidence of hypercellularity associated with an inflammatory phenotype was significant at early time points (12 weeks) in the traditionally processed bovine implants, which subsided with time. Conversely, bone in-growth into the implant was lowest in the traditionally processed bovine bone implants at 12 weeks, which increased to levels comparable to the other groups at 24 weeks. Taken together with the data presented above, it appears that residual donor material in the implants (expected in the traditionally processed bovine bone) evoked an inflammatory immune response, which adversely affected bone in-growth into the implants. This response subsided with time, at which time remodeling efficiency in these implants was able to approach levels seen in controls (allografts). On the other hand, BioCleanse® processed bovine xenografts by virtue of depleted donor residues, was able to be remodeled at a pace comparable to allografts.

In conclusion, results from these studies demonstrate that residual donor material can influence bone graft remodeling in this aggressive xenograft (bovine bone implants in sheep) model. Treatment of bovine bone by the BioCleanse® Tissue Sterilization Process, with its known ability to remove residual donor material, rendered these xenografts to remodel comparably to allograft.

4. Pain and loss of function

Pain and loss of function are risks to health associated with any implanted spinal device. Some device-related complications that may cause pain and loss of function include device fracture, deformation, loosening, extrusion, or migration due to inappropriate patient or device selection. The wear of some materials (i.e., polymeric materials), which may cause osteolysis (dissolution of bone), and component disassembly, fracture, or failure may also result in pain and loss of function.

To ameliorate these risks, guidance document "Spinal System 510(k)s" (May 3, 2004) recommends the use of labeling as well as mechanical testing, wear testing, animal testing, clinical testing as applicable.

Mechanical Testing

There are two widely accepted compendial test methods that outline the protocols for mechanical testing of spinal constructs. The two test methods are:

- ASTM F2077-03 – "Test Methods For Intervertebral Body Fusion Devices"
- ASTM F1717-04 – "Test Methods For Spinal Implant Constructs in a Vertebrectomy Model"

Both of these test methods prescribe detailed procedures for determining the *in vitro* biomechanical behavior of spinal hardware and/or implants. The Spinal System 510(k)s guidance document recommends the use of ASTM F1717 to allow a declaration of conformity to that standard. However, the guidance also states that we may choose alternative test methods that we show are substantially equivalent to the ASTM standard to address the mechanical loading issues at hand.

While both standards were reviewed with respect to the Sterling® Impacted Cortical Ring, it was determined that ASTM F2077 was more applicable to the design and performance characteristics of the construct. The rationale for using ASTM 2077 relates to the design of the Sterling® Impacted Cortical Ring and to the biomechanical characteristics of its intended use as a vertebral body replacement.

1. Sterling Impacted Cortical Ring – The size and shape of the graft lends it self to the testing as outlined in F2077, whereas F1717 specifically lists the test material design to consist of "screws, hooks, rods and transverse interconnections."

2. Absence of rods, screws, plates, etc. – The Sterling® Impacted Cortical Ring does not contain any instrumentation, fixation or attendant hardware. ASTM 1717 specifically calls out such hardware as being representative of the assembly to be tested.

3. Biomechanical Properties – The Sterling® Impacted Cortical Ring is intended to be placed in the thoracolumbar region of the spine where the biomechanical property characterization is served by the prescribed testing in ASTM F2077. Specifically the tests of Static Axial Compression, Dynamic Axial Compression and Torsion are outlined in F2077 for

implants of a design similar to the Sterling® Impacted Cortical Ring. While ASTM F1717 also outlines some of these tests, F1717 is more specifically directed toward screws, rods, assembled hardware and the interconnections between hardware items.

The Sterling® Impacted Cortical Ring was tested using ASTM F2077 because the test method was seen to be more applicable to the implant's design and construction whereas ASTM F1717 was determined to be applicable to spinal hardware consisting of screws, rods, hooks, and transverse interconnections.

Mechanical testing of the STERLING® Impacted Cortical Ring was performed in accordance with FDA guidance "Spinal System 510(k)s", issued May 3, 2004. Table 3b in Section 4 of this guidance document recommends the following tests for product code MQP:

- Static and dynamic axial compression bending testing
- Static and dynamic torsion testing
- Expulsion

For the dynamic axial compression bending tests, the guidance recommends that we meet one of the following conditions:

- asymptotic load level ≥3000N (~2x the vertebral body compression strength) at 5×10^6 cycles
- asymptotic load level ≥1500N (~1x the vertebral body compression strength) at 10×10^6 cycles

The study overview presented below provides evidence that the STERLING® Impacted Cortical Ring meets the special controls required by the FDA guidance.

The bovine ring xenograft implant is composed of a ring-shaped piece of cortical bone with convex superior and inferior surfaces and a footprint geometry wider than it is deep. The design allows the graft to be inserted using an anterior, oblique, or lateral approach. The graft is intended to be primarily used with commonly available insertion and impaction instruments which distract the vertebral bodies and deliver the graft with minimal, if any impaction.

Dimensionally, the graft footprint is 27 x 21 mm ± 2 mm (with M/L dimensions between 2 and 6 mm larger than A/P dimensions) in heights of 10-20 mm (in 2 mm increments) at the root of the ½ mm grooves. Graft surfaces in contact with the host bony surfaces are ridged to prevent expulsion of the implant and the posterior portion of the implant is tapered at an 8° angle to promote lordosis of the lumbar spine. The implant will be processed through the BioCleanse Tissue Sterilization Process, freeze-dried and terminally sterilized via STERRAD or gamma irradiation. The tested sizes for this report were: 10mm height (smallest) and 20mm height (tallest).

Mechanical Testing – Rationale for Sample Size Selection (worst case):

Compression Testing

Theoretically, the compressive stress is equal to applied compressive force divided by the cross-sectional area of the specimen. $\sigma = \frac{F}{A_c}$, where F is the applied axial force and A_c is the cross-sectional area. Another consideration must also be made, that of bending. A thin-walled cylindrical sample will tend to bend as it is compressively loaded due to bending in the sample.

Height: A taller sample would result in a higher likelihood of bending.

Sample Selection: H=20mm

Torsional Testing

Torsional shear stress, τ, is defined as $\tau = \frac{Tr}{J}$, where T is the applied torque, r is the radius at which the torsional shear is measured, and J is the polar moment of inertia of the specimen. If we manipulate this relationship, we obtain the following: $T_{max} = \frac{\tau_{max} J}{r_{outer}}$.

For an annular cross section, $J = \frac{\pi}{2}\left(r_{outer}^4 - r_{inner}^4\right)$. Note that $\tau_{max} = G\gamma_{max}$ and $\gamma_{max}\ \alpha\ \frac{1}{H}$.

From these relationships, inference can be made to obtain a worst case specimen.

Height: By increasing the height, H, of the specimen we increase the shear strain observed on the cortical bone ring and thus reduce the max shear strain of the material. Thus, the taller cortical bone ring would fail under a smaller torque than a shorter sample.

Sample Selection: H=20mm

All samples were subjected to impact testing (10 cycles of 700 lbf / 3110N) prior to further testing. The testing of impacted samples further represented a worst case milieu in that it is unlikely that individual grafts would have been subjected to as many as ten impacts in a clinical setting.

Mechanical Testing – Results:

Table 2: Summary of Mechanical Testing Acceptance Criteria and Results

	TEST						
Sample Size (height)	**Impact** (10 cycles of 700 lbf each)	**Static Axial Compression**		**Dynamic Axial Compression** (run out at 5 X 10^6 cycles)		**Torsion (ultimate torque)**	**Expulsion**
		Acceptance Criterion	Result	Acceptance Criterion	Result	Informational Test Results	Informational Test Results
10mm	passed	>9980N	42,800N	4440N	5782N	Not tested*	431N
20mm	passed	>9880N	42,600N	4440N	5656N	19.3 Nm	Not tested*

*Not Tested: for torsion: 20mm is worst case; for expulsion: 10mm is representative

In each case, the Sterling Impacted Cortical Ring met or exceed acceptance criteria. Where informational testing was performed, no untoward results were noted. For dynamic compression it should be noted the values listed in the table above are the smallest values for the maximum compressive loads for 5 million cycles. The biomechanical testing results of the xenograft cortical ring spacer are acceptable within the testing regimen and protocols of this study and the results support the use for which it is designed.

Mechanical Testing – Materials Characterization Study

Samples of BioCleanse®-treated cortical bovine bones were tested, measuring axial compression strength, diametral compression strength, shear, three point bending strength, and residual moisture. BioCleanse®-treated cancellous bovine bone was tested for axial compression strength.

The mechanical testing that generated axial compression, diametral compression, shear, three point bending, cancellous plug axial compression, and residual moisture data utilized specimens designed specifically for those tests. Samples of BioCleanse®-treated cortical bovine bones were tested, measuring axial compression strength, diametral compression strength, shear, three point bending strength, and residual moisture. BioCleanse®-treated cancellous bovine bone was tested for axial compression strength.

Table 3: Specimens tested for material property characterization at time zero (T_0)

Test	**Sample Size**	**Average ± StDev (MPa)**	**Range (MPa)**
Axial compression	35	165 ± 42	86 - 244
Diametral compression	35	30 ± 8	12 - 45
Shear	34	57 ± 11	37 - 82
Three point bending	8	147 ± 16	121 - 169
Cancellous plug axial compression	33	3.43 ± 2.84	0.36 - 9.24

Table 4: Specimens tested for material property characterization at one year accelerated aging (T_1)

Test	Sample Size	Average ± StDev (MPa)	Range (MPa)
Axial compression	35	176 ± 43	83 - 249
Diametral compression	31	26 ± 10	9 - 53
Shear	33	68 ± 12	39 - 96
Three point bending	8	159 ± 31	109 - 216
Cancellous plug axial compression	31	2.61 ± 3.52	0.16 - 16.75

Table 5: Specimens tested for material property characterization at one year real-time aging (T_2)

Test	Sample Size	Average ± StDev (MPa)	Range (MPa)
Axial compression	33	203 ± 39	101-254
Diametral compression	36	29 ± 7	14-40
Shear	34	83 ± 13	53-107
Three point bending	8	129 ± 34	85-179
Cancellous plug axial compression	33	4.28 ± 6.78	0.21-28.26

Table 6: Specimens tested for material property characterization at three years accelerated aging (T_3)

Test	Sample Size	Average ± StDev (MPa)	Range (MPa)
Axial compression	35	214 ± 40	102-284
Diametral compression	35	25 ± 8	10-40
Shear	34	57 ± 13	34-98
Three point bending	7	122 ± 13	106-146
Cancellous plug axial compression	33	4.25 ± 3.48	0.22-11.41

Wear Testing

Bovine cortical bone is a natural material that is similar in composition and biomechanical behavior to human cortical bone, such as would be found in the clinically prevalent Femoral Ring Allograft (FRA). As such it is not a novel material that would raise issues of particulate generation, but rather it is a natural analog to FRA (see Clinical Literature Review below).

Rat and Sheep Materials Implant Studies

The Spinal System 510(k)s guidance comments that animal studies are typically conducted to evaluate a biological response to a new material in the spine or to evaluate the functional behavior of a device system. The Sterling Impacted Cortical Ring is manufactured from a material currently FDA-cleared for used in the spine (i.e., bovine bone processed in the same manner as the Sterling Cancellous Cubes, K051615). Local and systemic adverse effects were evaluated through extensive materials biocompatibility and immunotoxicity testing as described in Section 4 above. Because of this testing, questions of device performance can be adequately answered by mechanical testing alone.