Pathology Report

Study Title: An *In Vivo* Study Using an Ovine Cervical Fusion Model – Histopathology of Non-Target Tissues

An *in vivo* study using an ovine cervical fusion model was performed at the AHN Research Institute under the direction of Boyle Cheng (Principal Investigator).

Fourteen mature sheep were used in the study. Seven of the sheep were sacrificed at 12 weeks post-surgery. Seven sheep were sacrified at 26 weeks post-surgery. The focus of the study was the evaluation of intervertebral disk devices (test articles). A report describing the pathology surrounding and associated with these intervertevral disks has previously been submitted. **The present study evaluated the histopathologic appearance of the non-target tissues that were collected at the time of animal sacrifice.**

The non-target tissues collected:

1. Axilllary lymph nodes
2. Inguinal lymph nodes
3. Periaortic lymph nodes
4. Mesenteric lymph nodes
5. Liver
6. Heart
7. Spleen
8. Pancreas
9. Lungs
10. Kidneys
11. Spinal Cord Tissue

Images were also acquired from the slides that contained 50µ thick sections of the test articles and adjacent bony and fibrous tissue in an attempt to identify the cell types present in the adjacent tissue. However, the extent to which this was possible was limited by the thickness of the sections and the refractivity of the methacrylate and glass slides.

Glass slides of the non-target tissues submitted for examination were stained with hematoxylin and eosin (H&E). A semiqunatitative evaluation of these tissues was provided in a report submitted by Alizée Pathology, LLC (Thurmont, MD 21788).

The findings of the present study largely confirm the findings reported by Alizée Pathology, LLC with respect to the non-target tissues. In brief summary, there were no inflammatory, degenerative, neoplastic or infectious processes noted in any of the non-target tissues from any of the animals. The present report documents these results below and provides representative images of the selected tissues.

Individual Organ Reports:

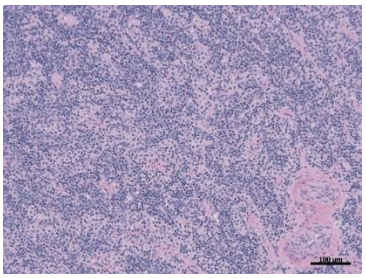
1. Axilllary lymph nodes: Histopathologic examination showed normal lymphoid tissue with non-reactive cortical follicles and a normal medullary component.
2. Inguinal lymph nodes: Histopathologic examination showed normal lymphoid tissue with non-reactive cortical follicles and a normal medullary component.
3. Periaortic lymph nodes: Histopathologic examination showed normal lymphoid tissue with non-reactive cortical follicles and a normal medullary component.

4. Mesenteric lymph nodes: Histopathologic examination showed normal lymphoid tissue with non-reactive critical follicles and a normal medullary component.
5. Liver: Hepatic microarchitecture was observed to be normal with no evidence for inflammation, fibrosis, degeneration, or neoplasia. The central veins and portal triads were normal in cellularity and architecture.
6. Heart: Histopathologic examination of myocardial tissue was unremarkable. No evidence for inflammation, degeneration, or other pathology was noted.
7. Spleen: The spleen showed an abundant red pulp component with a normal distribution of lymphoid tissue and a normal fibrous capsule. No evidence of inflammation was seen.
8. Pancreas: Histopathologic examination of the pancreas showed normal lobular architecture with scattered islets throughout the exocrine component of the pancreas.
9. Lungs: Histopathologic examination of the lungs showed normal bronchial, bronchiolar, and parenchymal architecture. There is no evidence for inflammation, edema, congestion, or other pathologic findings. Occasional small areas of atelectasis were present.
10. Kidneys: Histopathologic examination showed a normal cortical and medullary component. No evidence for inflammation or other pathologic findings was seen.
11. Spinal Cord Tissue: Sections of cervical spinal cord tissue showed no evidence for inflammation, degeneration, or other significant pathologic findings.

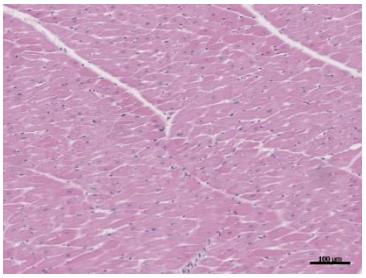
Representative images of the tissues from 3 of the animals (#6084, #6085, and #6086) are provided below with a brief description of the findings for each image.

Representative images of the tissues harvested from all animals provided in the attachment at the end of this report

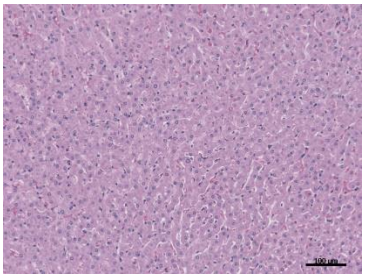
AZ496 EJG17-438 6084: Cervical lymph node shows a normal number and distribution of lymphocytes.



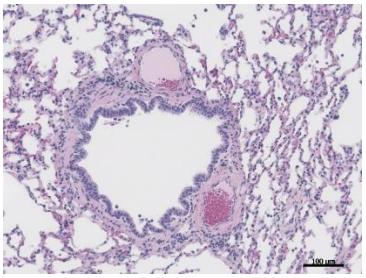
AZ496 EJG17-438 6084: Heart tissue shows normal histologic appearance.



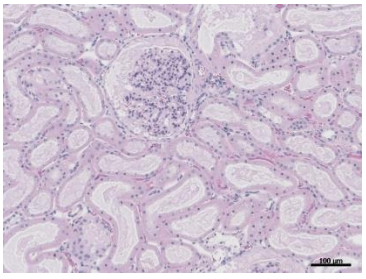
AZ496 EJG17-438 6084: Liver shows normal appearance.



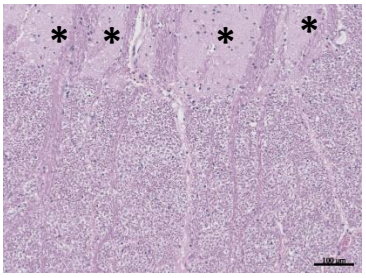
AZ496 EJG17-438 6084: Lung tissue shows cross section of bronchiole and surrounding normal pulmonary parenchyma.



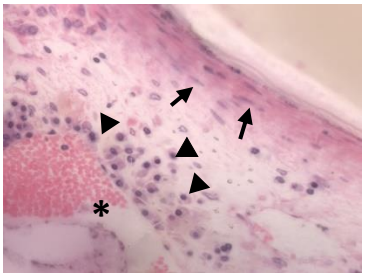
AZ496 EJG17-438 6084: Renal tissue shows a normal glomerulus surrounded by normal renal tubules.



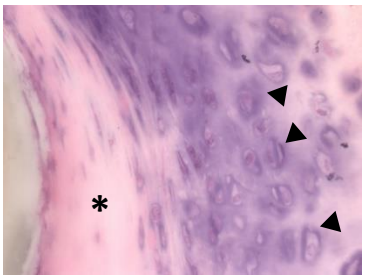
AZ496 EJG17-438 6084: Spinal cord tissue at C4 shows normal appearance of white matter (spinal cord tracts) and border of central grey matter (asterik).



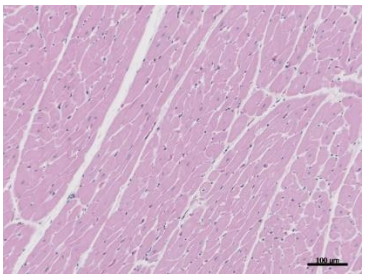
AZ496 EJG17-438 6084: Interface of host tissue with Xiphos IVD device. Host tissue shows fibrous band with elongate fibroblasts (arrows), lymphocytes (arrowheads), and a venous vascular structure (asterik).



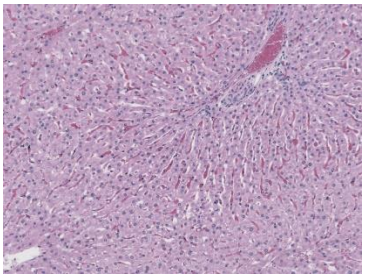
AZ496 EJG17-438 6084: Interface of host tissue with ZFuze IVD device (left side of image). Host tissue shows fibrous connective tissue at the interface (asterik) with adjacent osteoblasts (arrowheads) embedded within osteoid.



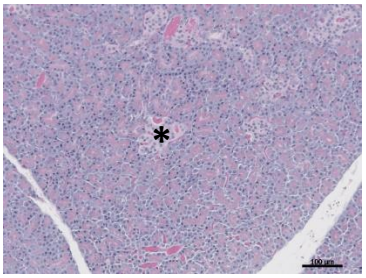
AZ496 EJG17-438 6085: Heart tissue shows normal morphology.



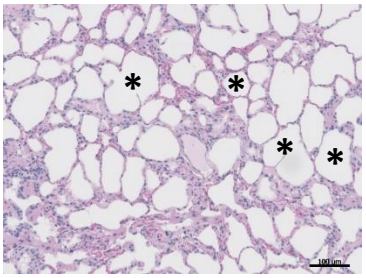
AZ496 EJG17-438 6085: Liver shows normal morphology.



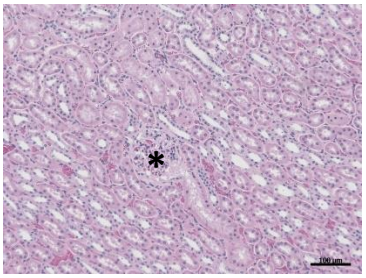
AZ496 EJG17-438 6085: Pancreas shows normal islets (asterik) and normal exocrine pancreatic parenchyma.



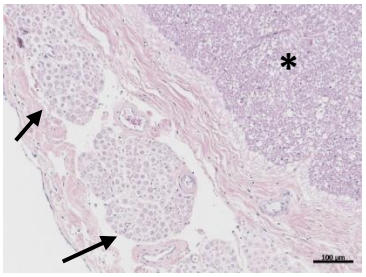
AZ496 EJG17-438 6085: Lung tissue shows normal parenchyma with clear alveolar spaces (asteriks) and normal interstitium.



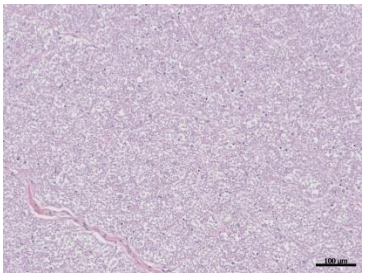
AZ496 EJG17-438 6085: Kidney shows normal renal cortex with glomerulus (asterik) and normal proximal renal tubules.



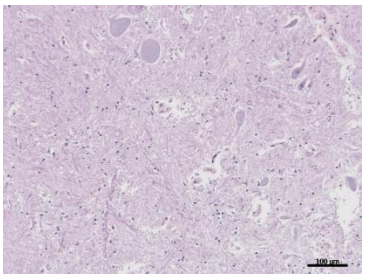
AZ496 EJG17-438 6085: Spinal cord tissue near C2-C3 with ZFuze IVD device shows normal white matter (asterik) and adjacent spinal nerves (arrows).



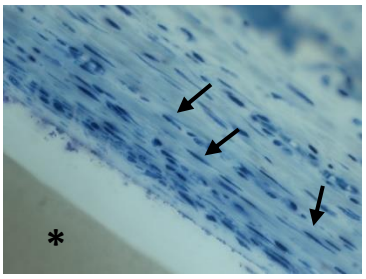
AZ496 EJG17-438 6085: Cross section of spinal cord near C2-C3. Histology shows normal morphology. ZFuze IVD device at this disk space.



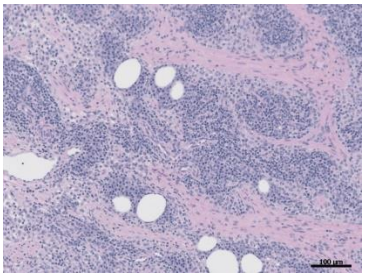
AZ496 EJG17-438 6085: Histologic examination of white matter in spinal cord at C2-C3 shows normal apperance. ZFuze device placed in C2-C3 IVD space.



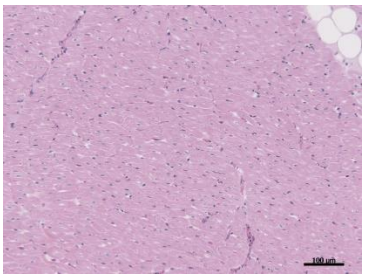
AZ496 EJG17-438 6085: Interface of ZFuze device (asterik) and adjacent host tissue that consists of spindle-shaped fibroblasts (arrows) and collagenous connective tissue.



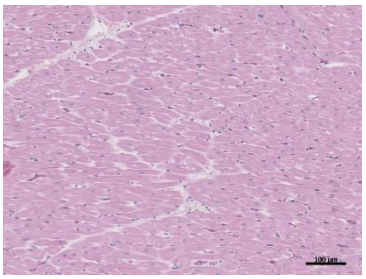
AZ496 EJG17-438 6086: Cervical lymph node shows normal distribution of lymphocyte (dark staining cells) and stroma (pink staining tissue).



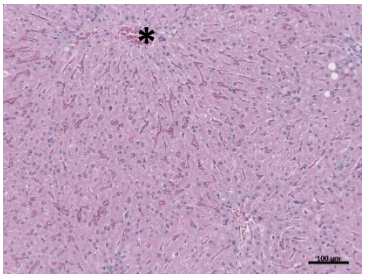
AZ496 EJG17-438 6086: Heart shows normal histomorphology.



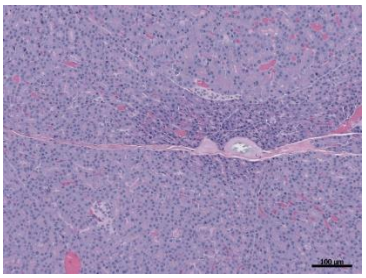
AZ496 EJG17-438 6086: Normal myocardial morphology is depicted.



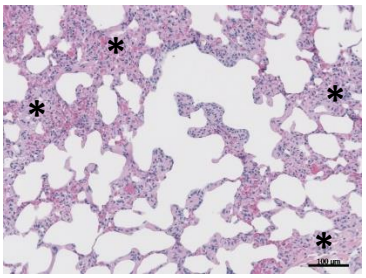
AZ496 EJG17-438 6086: Liver shows normal morphology. Central vein of hepatic lobule (asterik).



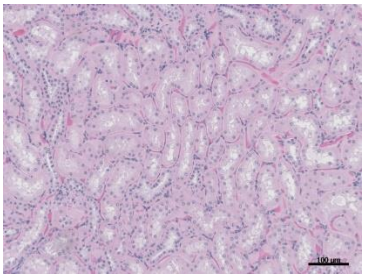
AZ496 EJG17-438 6086: Normal pancreatic histomorphology.



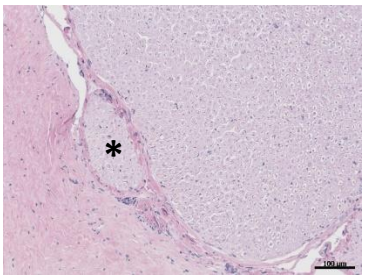
AZ496 EJG17-438 6086: Lung tissue shows mild atelectasis (asterik) and clear alveolar spaces.



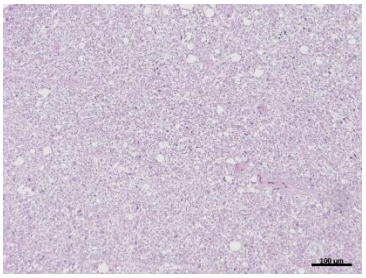
AZ496 EJG17-438 6086: Kidney shows normal tubular architecture and morphology.



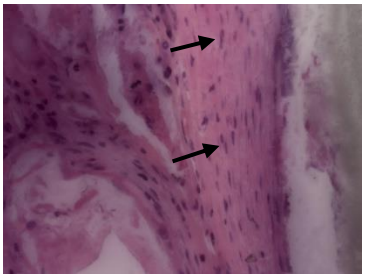
AZ496 EJG17-438 6086: Spinal cord at approximately C2-C3 shows normal white matter with associated spinal nerve (asterik).



AZ496 EJG17-438 6086: Spinal cord at C2-C3 shows normal white matter. Xiphos IVD device implanted at C2-C3.



AZ496 EJG17-438 6086: Interface of ZFuze IVD device and host tissue. Thin band of fibrous tissue with elongate fibroblasts (arrows) adjacent to ZFuze device.



Conclusion: The implantation of the test articles was not associated with any peripheral tissue pathology in this animal model.

I have reviewed the final report of Alizee Pathology with respect to the Ovine cervical fusion study and found that the report conforms to the intent, guidelines and standards of ISO-10993-6. The report concludes that there is no biologically significant difference between the host response to the test article and the control article.

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