Recommended Procedure for HD-XG to Determine the Correct Insertion Depth for Mouse Carotid

To maintain sensor patency throughout the length of a study, the sensor tip should be located in the freely-flowing blood of the aortic arch. Depending on the mouse strain, gender, age and size (weight), the sensor may have to be advanced to different depths. It is recommended, before any survival surgery is performed, to determine the sensor insertion depth for each type of mouse to be implanted. This is done by performing a sternotomy on at least two representative euthanized mice and locating the aortic arch in relation to the carotid bifurcation. More animals may be necessary if there is a high degree of variability in the animal population. Measurements are taken to determine the appropriate length to insert the sensor. Follow the example below for exact steps.

Note: This process must be performed for every cohort, strain, gender and weight including genetically modified models. See end of document for more details on genetically modified or inbred strains of mice.

Example: Implanting a cohort of 30 C57 male mice.

1) Weigh all of the mice and put the weights in order of the smallest to the largest.

2) Determine the mouse whose weight is the middle (#15 highlighted in yellow). That mouse will be the median mouse.

3) Now identify the animal whose weight is midway between the smallest mouse and the median mouse (#8 highlighted in green). This will be the first animal to be used for a depth measurement.

4) Now identify the animal whose weight is midway between the median mouse and the largest mouse (#22 highlighted in pink). This will be the second animal to be used for a depth measurement.

5) Anesthetize one of the animals to be measured. Clip the hair from the ventral neck and chest area.

6) Isolate the carotid bifurcation (internal and external carotid). Isolating prior to euthanizing the animal makes the carotid bifurcation easier to identify.

7) Tie a piece of suture at the bifurcation. (See Figure 1)

Animal #	Weight (g)
1	28.7
2	28.8
3	28.9
4	28.9
5	29.4
6	29.5
7	29.8
8	30.3
9	30.6
10	31.1
11	31.3
12	31.3
13	31.4
14	31.4
15	31.4
16	31.6
17	31.8
18	32.1
19	32.1
20	32.2
21	32.4
22	32.4
23	32.5
24	32.6
25	32.7
26	32.9
27	33
28	33.5
29	34.6
30	34.7

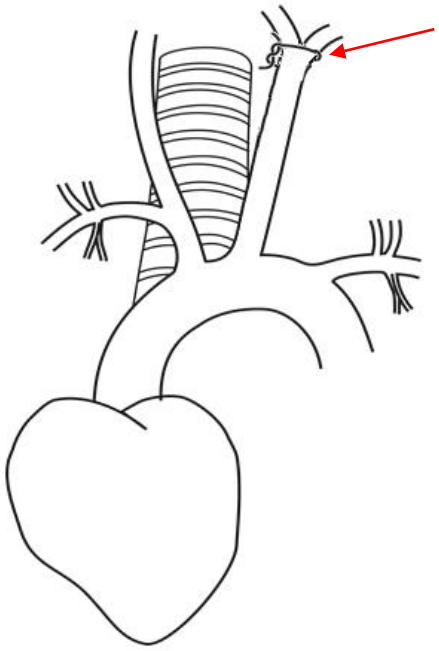

Figure 1: Suture tie placed at carotid bifurcation

8) Euthanize the animal by a standard technique. **Do not use cervical dislocation because this can change your measurements.** Place the animal in dorsal recumbency with the hindlimbs near you and the head away from you.

9) Using a small surgical scissors, cut across the diaphragm and through the rib cage on both the right and left lateral sides and remove the chest plate to fully expose the thoracic cavity. (See Figure 2)

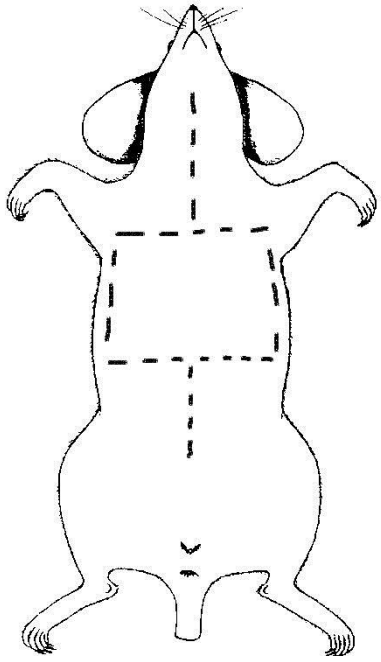

Figure 2: Incisions for locating the carotid and aortic arch

10) Along the midline cut through the manubrium and incise cranially to the ventral neck region. (See Figure 2)

11) Locate the heart. The thymus will first need to be dissected before the aortic arch can be visualized. The thymus is white in color and can be gently removed. **Be careful to only grasp the thymus during the dissection. Avoid grasping any vessels as the aortic arch is located underneath the thymus.**

12) Once the thymus is removed, locate the aortic arch. Gently isolate the arch from the surrounding tissue.

13) Locate where left carotid artery branches from the aortic arch.

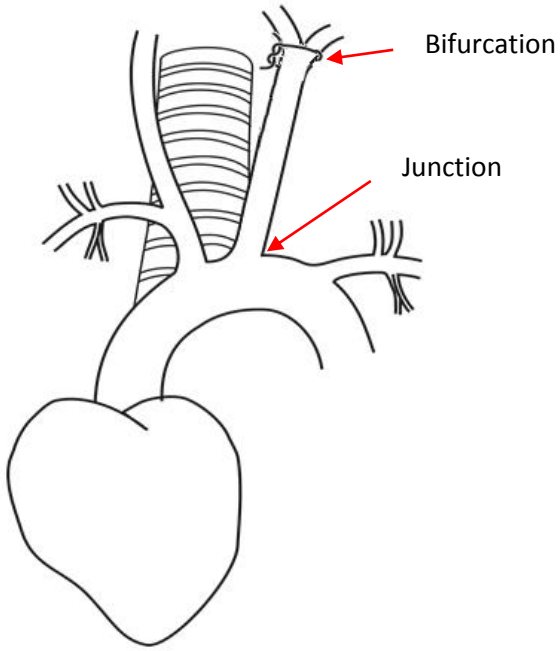

Figure 3: Suture tie at carotid bifurcation and junction of left carotid artery to aortic arch

14) Locate the left carotid artery and the suture at the bifurcation. (Figure 3)

15) Measure in millimeters from the carotid bifurcation suture to the junction of the carotid artery and aortic arch. Record this measurement. (Figure 4)

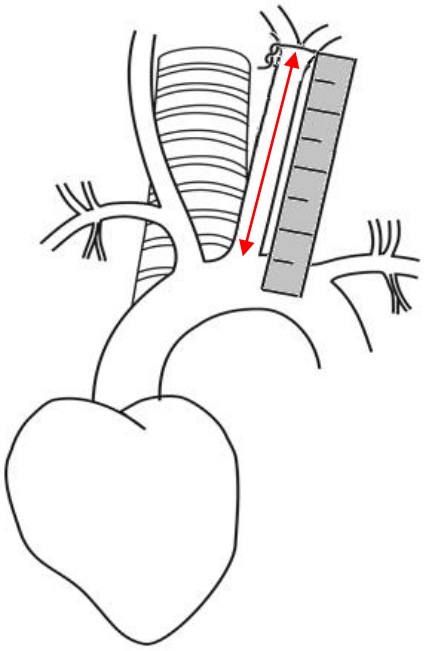

Figure 4: Measure from suture to top of arch

16) Repeat steps 5 through 16 for the second representative mouse.

17) Optimal placement should be with 2 mm of the sensor positioned in the aortic arch. (See Figure 5)

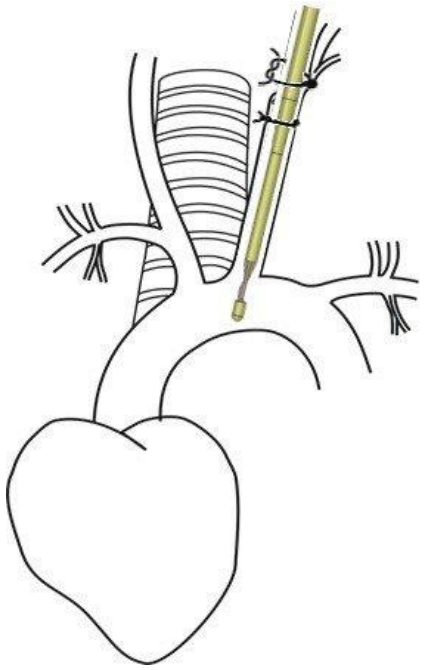

Figure 5: Sensor placement of 2 mm in the aortic arch

18) Since placement within the aortic arch is desired, add an additional 2 mm to the measurement of the distance between the carotid artery bifurcation suture and the top of the aortic arch to determine the total insertion depth.

    a. Desired placement is 2 mm into the aortic arch:
        i. If your total insertion depth was 14 mm and the white marker band was located at 12 mm then the sensor would be advanced until the marker band is 2 mm caudal to the bifurcation suture.
            1. Total depth 14 mm = 12 mm carotid artery bifurcation to the top of the aortic arch + 2 mm

        ii. If your total insertion depth was 12 mm and the white marker band was located at 12 mm, then the sensor would be advanced until the marker band is even with the bifurcation suture. (See Figure 6)
            1. Total depth 12 mm = 10 mm carotid artery bifurcation to the top of the aortic arch + 2 mm

Note: Each sensor must be measured as there will be slight variation in the location of the white marker band.

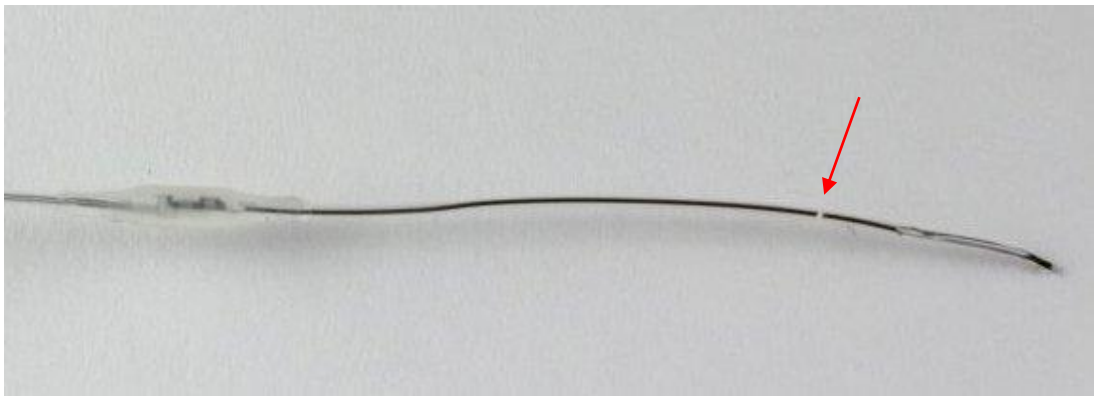

Figure 6: White marker band on sensor

19) Repeat this process when changing to a new cohort of animals, strain, gender or weight range of animals.

20) After determining the correct depth for your representative mice from above, use these animal weights and measurements to determine the depth for the rest of the cohort.

    a. Record all your measurements and the depth used for each animal. As a general guide add or subtract approximately 0.5 mm for every 1 gram of body weight difference.

    b. Even though you may use the 0.5 mm as a guide as stated above it is important to make sure that the depth that you select it is still in line with the two representative mice that you measured. At a certain age animals grow more in girth than in length so there will be a maximum depth that you should never exceed. Experience will allow you to determine this depth.

    c. Example:

Animal #	weight	Measured depth	add 2 mm
1	28.7		11
8	30.3	10	12
9	30.6		12
15	31.4		12.5
21	32.4		13
22	32.4	11	13
29	34.6		14

Note: These measurements are for the example only. The cohort of animals that you will use will be different than these above. Do not use these measurements for your own animals.

21) When first working with the sensor, verify tip placement following the Verification of Sensor Placement section in the Appendix in the surgical manual on all implanted animals. This will allow a better determination of depth and feedback on surgical technique.

    a. If the sensor was inserted 12 mm by the surgeon and upon necropsy the sensor was found to only be inserted 10 mm, the surgeon ought to take extra care to ensure measurements are correct in future surgeries and that the sensor is not slipping back while the surgeon is tying the knots. The surgeon may also wish to ensure knot-tying technique is correct and square knots are tied securely.

22) If the depth placement is consistently very successful after working with the sensor for a while, only perform the verification in the case of data that is not as expected.

23) If all the animals except for a few worked well, do not make adjustments to your technique for depth measurements. **The depth determination process needs to be completed with every cohort as over time the genetics can change and the measurements will change.**

24) If most of the animals had issues with the data, adjust your depths based on the animals that were explanted and had tip placement verified. Necropsy data is very important to determine the potential causes of failures and if any changes are needed to improve placement of the sensor. Refer to the Verification of Sensor Tip Location section in the Appendix.

25) By verifying sensor tip placement, adjustments can be made, if needed, for subsequent surgeries involving the same strain, gender and weight of animal.

26) Contact Technical Support with any questions at support@datasci.com.

Genetically Engineered and Inbred Mice

Due to the high value of these animals, it is understandable that it is not practical to sacrifice the animals to obtain a depth measurement. However, measurements must be taken to determine the correct depth to ensure accurate data. Use an animal from a terminal study that is in the same weight, sex and strain as the ones you will be implanting. If this is not feasible, implant only one animal at a time verifying the tip location and adjust the measurements as you have more experience.