These procedures must be followed by euthanasia as it is only permissible as a terminal procedure unless it is scientifically justified and approved in an IACUC protocol.

Intracardiac puncture MUST be done UNDER ANESTHESIA.

1. **RAT INTRACARDIAC PUNCTURE PROCEDURES**
   1.1 Intracardiac Puncture with Rat in Dorsal Recumbency

   1.1.1 Anesthetize rat (according to approved protocol)
   1.1.2 Place rat in dorsal recumbency
   1.1.3 Prep area just below sternum with alcohol swab.
   1.1.4 Insert needle at base of sternum on a 20 degree angle under or just left to the xyphoid process (Images 2 & 3)
   1.1.5 Aspirate syringe slowly while advancing needle
   1.1.6 Stop needle advance when blood is visualized in syringe tip
   1.1.7 Hold needle and syringe steady until all blood is collected
   1.1.8 Perform a bilateral thoracotomy with scissor or a scalpel to insure death
1.2 Intracardiac Puncture with Rat in Right Lateral Recumbency

1.2.1 Anesthetize rat (according to approved protocol)
1.2.2 Place rat in right lateral recumbency
1.2.3 Prep area behind forelimb with alcohol swab.
1.2.4 Insert needle just behind the forelimb (Image 4) where the heartbeat is easily felt. The location of the heart is approximately under the elbow when leg is placed on a normal anatomic position (Image 1)
1.2.5 Aspirate syringe slowly while advancing needle
1.2.6 Stop needle advance when blood is visualized in syringe tip (Image 4)
1.2.7 Hold needle and syringe steady until all blood is collected
1.2.8 Perform a bilateral thoracotomy with scissors or a scalpel to insure death

2. **MOUSE INTRACARDIAC PUNCTURE PROCEDURE**

2.1 Anesthetize mouse (according to approved protocol)
2.2 Place mouse in dorsal recumbency
2.3 Prep area just below sternum with alcohol swab.
2.4 Insert needle at base of sternum on 20 degree angle under or just left to the xiphoid process (Image 1)
2.5 Aspirate syringe slowly while advancing needle
2.6 Stop needle advance when blood is visualized in syringe tip
2.7 Hold needle and syringe steady until all blood is collected (Image 2)
2.8 Perform a bilateral thoracotomy with scissors or a scalpel to insure death