I PURPOSE

The purpose of exsanguination is to humanely sacrifice the piglet and also collect biological fluid and organ samples.

II SCOPE

Isoflurane is used to exsanguinate the piglet. Maintaining adequate blood flow during collection maximizes the amount of blood to be collected and analyzed. Pictures of samples stored on ice and the specimen mixer are shown.

III RESPONSIBILITIES

It is the responsibility of Metabolic Assessment Laboratory personnel to follow this procedure. It is the responsibility of supervisory personnel to ensure compliance with this procedure and to train employees and students responsible for performing this procedure. Students will report accidents to the principal investigators immediately.

IV REFERENCES

N/A

V REAGENTS AND MATERIALS

V.A. Anesthesia apparatus (mask, oxygen regulator, isoflurane to exsanguinate the piglet).
V.B. Vacutainer tubes labeled for biological fluid (portal, central blood).
V.C. Vacutainer collection tube.
V.D. 20 gauge Vacutainer needle to draw central blood.
V.E. 22 gauge Vacutainer needle to draw portal blood.
V.F. 5cc syringe with 22-gauge needle for urine.
V.G. Scalpel holder with appropriate size blade (size 10).
V.H. Assortment of small (11cm) and medium (14cm) scissors.
V.I. Assortment (8cm-12cm) of toothed forceps.
V.J. Paper towels.
V.K. Ice.
V.L. 20 mL scintillation vials labeled for urine.
V.M. 70% Ethanol.
V.N. Black Ice Buckets.

VI EQUIPMENT

VI.A. Specimen mixer to invert blood tubes.
VII SAFETY PRECAUTIONS

VII.A. Members of the MAL have been trained extensively in the procedures described in this SOP.

VII.B. Member of the MAL have completed animal contact training in order to work in the PNICU.

VII.C. Members of the MAL have been approved to work with human blood and piglet blood, tissues, and urine.

VIII DEFINITIONS

VIII.A. Standard Operating Procedure (SOP) – Standard Operating Procedure is a document that provides instructions for completing a specific task in the lab.

VIII.B. Metabolic Assessment Laboratory (MAL) – The Metabolic Assessment Laboratory is the laboratory that will use this SOP.

VIII.C. Piglet Restraints – Thin strips of cloth material used to tie the piglet’s limbs down and away from the surgeon.

VIII.D. Piglet Neonatal Intensive Care Unit (PNICU) – The PNICU is a unit where the piglet is monitored by 24 hour care and routine check-up parameters using PNICU SOPs conducted by the MAL.

VIII.E. Piglet V-Holder—The Piglet V-Holder is a metal basin that is V-shaped and supports piglet’s body during surgery.

VIII.F. Piglet Facility—The Piglet Facility is comprised of three rooms: the PNICU used for piglet care; a surgery room used for surgical procedures; and a transition/entry room used by staff to prepare items for care or surgery and to transfer the piglet from surgery to care.

VIII.G. Clean Person—The Clean Person is designated to open and touch non-sterile items to maintain the integrity of the sterile person and surgeon.

VIII.H. Sterile Person—The Sterile Person is designated to handle all sterile instruments and materials that will be used inside the piglet. The Sterile Person also passes instruments to the surgeon.

IX PROCEDURE

IX.A. Procedure for anesthetization of the piglet

IX.A.1. Actions performed by the anesthesiologist as the piglet is being prepared for surgery in the entry room.

IX.A.1.a. Turn on the oxygen regulator until the level is 5.

IX.A.2. Actions performed as piglet is being prepared for anesthesia.

IX.A.2.a. Apply the anesthesia mask to the piglet’s snout.

IX.A.2.b. Administer 5% isoflurane by turning the valve immediately afterwards.
IX.A.2.b.(1). Make sure the mask is held to the piglet’s face.
IX.A.2.b.(2). It may take longer to anesthetize a large piglet.
IX.A.2.b.(3). Monitor the color and tidal volume of the piglet.
   Follow directions given by the surgeon for controlling
   the level of isoflurane.
IX.A.2.b.(4). Hand Dr. Borum piglet restraints and tape to secure
   the piglet’s limbs to the Piglet V-holder.

IX.B. Initiation of the first incision
   IX.B.1. After washing the ventral side of the piglet with ethanol, the surgeon makes the
   first incision below the sternum using a scalpel.
   IX.B.1.a. The clean person records the time of first incision.
   IX.B.1.b. Participants assist the surgeon by cleaning up blood loss using paper
   towels.

IX.C. Central and/or portal blood may be collected
   IX.C.1. The surgeon uses a 20 gauge Vacutainer needle attached to a Vacutainer
   collection tube for the central blood collection from the left ventricle; a 22
   gauge Vacutainer needle is used for portal blood collection.
   IX.C.1.a. Participants may assist the surgeon by handing empty Vacutainer
   tubes.
   IX.C.1.a.(1). Clean person holds back the ribcage using toothed
   forceps as directed by the surgeon.
   IX.C.1.a.(2). To the right of Dr.Borum, the sterile person is handing
   empty Vacutainer tubes. This person should have
   plenty of vacutainer tubes in hand to minimize any
   delay.
   IX.C.1.a.(3). To the left, another member is receiving the filled
   tubes to hand to another member to place the tubes on
   the specimen mixer.
   IX.C.1.b. The surgeon hands Vacutainer tubes with whole blood to a
   participant to initiate specimen mixing.
   IX.C.1.b.(1). The Vacutainer tubes are manually inverted once.
   IX.C.1.b.(2). The Vacutainer tubes are then placed on the specimen
   mixer located on the mobile stainless working table
   (see Figure 1).
   IX.C.1.b.(3). Each tube of blood is rocked 15-20 times on the
   specimen mixer. One rotation is a back and forward
   motion of the mixer. Refer to Figure 1.
   IX.C.1.b.(4). After the tube of blood has been rocked 15-20
   rotations, place it in pre-made, vertical holes in the ice
   bucket on a working table.
   IX.C.1.b.(5). Multiple samples can be rocked simultaneously, but if
   this is the case extra caution must be taken to keep
   track of each individual sample’s count.
IX.C.1.b.(6). The bucket containing whole blood will be transferred to the lab where blood analysis will be performed according to SOP# CP001.00.

![Image of a bucket containing blood](image)

Figure 1.

IX.D. Urine collection
   IX.D.1. Have a 22-gauge needle affixed to a 5cc syringe laid out on the non-survival surgery tray.
   IX.D.2. The surgeon draws urine from the bladder of the piglet. If additional urine is available, only the syringe will be replaced. The needle will remain within the bladder.
   IX.D.3. Total urine volume is recorded by the clean person.
   IX.D.4. Urine is removed from syringe into 20mL white capped scintillation vials and stored on ice until it is transferred to the lab where urinalysis will be performed according to SOP# CP020.00.

IX.E. Upon collecting blood and urine, removal of organs may commence according to SOP# CP006.00.
X  ATTACHMENTS

X.A.  Layout of non-survival surgery instruments

X.A.1.  Non-survival surgery instruments are placed on towels on the operating table. Needles should already be affixed to their appropriate syringes (urine syringe).
X.B. The following layout for MAL personnel is suggested during blood and urine collection in the surgery room.

![Diagram of surgery room layout]

X.C. For a list of materials and their locations, refer to SOP# CG098.00.