MICE HANDS-ON EXERCISE:

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FOUNDATION FOR RESEARCH AND TECHNOLOGY- HELLAS

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- ✓Handling
- ✓Restraining
- ✓Sex Identification
- ✓Routes of Administration
- ✓Blood Sampling
- ✓Anesthesia
- ✓Euthanasia
- ✓Suturing

General biological and physiological data

- The laboratory mouse (Mus musculus) is a small rodent
- Most active at night (nocturnal animal)
- High metabolism:
  - Average body temperature: 37°C
  - Respiratory rate: 95-165 breaths/minute
  - Heart rate: 325-800 beats/minute
- Poor vision, acute sense of hearing and smell
- Daily water consumption: 5 ml
- Daily food consumption: 5 g
- Social animals (adult males may require separation if aggressive)

General biological and physiological data

- Sexual maturity: 6-7 weeks in females; 7-8 weeks in males
- Reproductive span: 7-9 months
- Estrus cycle length: 4-5 days
- Duration of estrus: 12 hours
- Average litter size: 6-10
- Average birth weight: 0.5-1.5 g
- Gestation period: 19-21 days
- Weaning age: 21-28 days

- Male adult weight: 25-40 g
- Female adult weight: 20-40 g
- Life span: 1.5-3 years

GENERAL CONSIDERATIONS

- Proper clothing
  - laboratory clothes
  - surgical mask
  - disposable gloves
  - hair net
  - shoe covers
- Prepare necessary material.
- Plan refined moves and procedures.
- Double-check animal/cage labeling.
- Be calm, quiet, gentle, concentrated.

HANDLING

- By the tail:
  - Used for brief restraint- cage transfer.
  - Easy to restrain but vulnerable to injury.
  - Gently pick it up from the base of tail, not tip!
  - Do not suspend mouse for prolonged periods.
  - If intimidated it will try to get away or bite!
- Small pups: Try cupping them!!

TYPES OF RESTRAINT

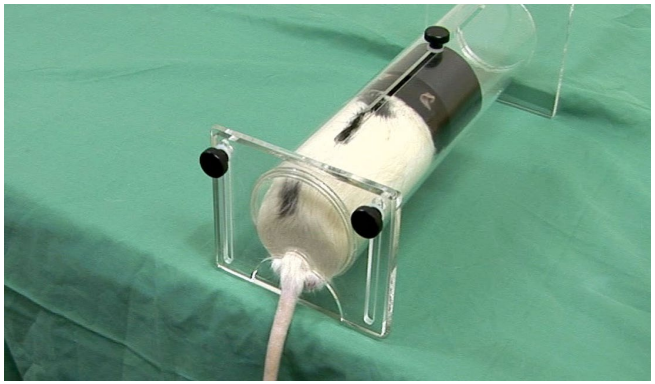
- One handed restrain.
- In a restrainer.
- In a tunnel.
- Cup handling.

ONE HANDED RESTRAIN

- Place on the cage top (foothold).
- They have a strong grip.
- If intimidated it will try to get away or bite!
- Scruff between thumb and forefinger.
- Grip tail between pinky. Secure.
- Used to perform minor procedures (injections, blood sampling, marking)

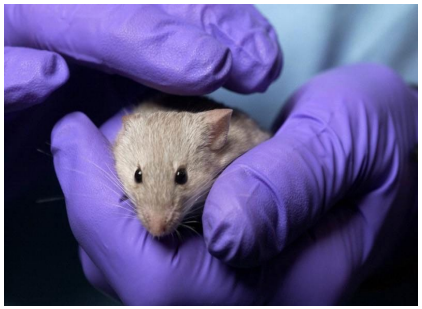
RESTRAIN IN A RESTRAINER

- Restrain manually and release in restrainer.
- Cover the top of the restrainer with one hand to form a dark tunnel.
- Secure device.
- Check status (breathing etc.).
- Gently return to cage.



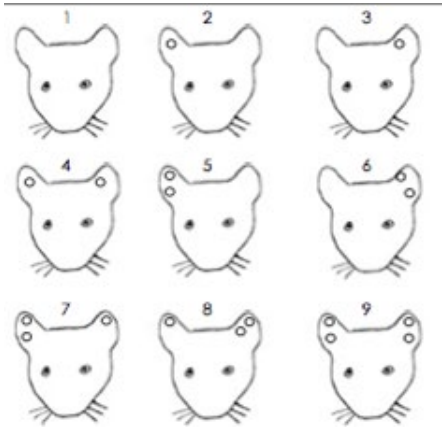
RESTRAIN IN A TUNNEL & CUP HANDLING

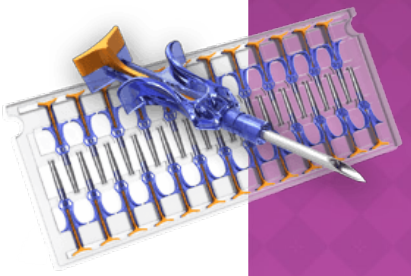
- Mice are more willing to interact voluntarily with the handler.
- Safer – non-aversive-less stressful handling methods.



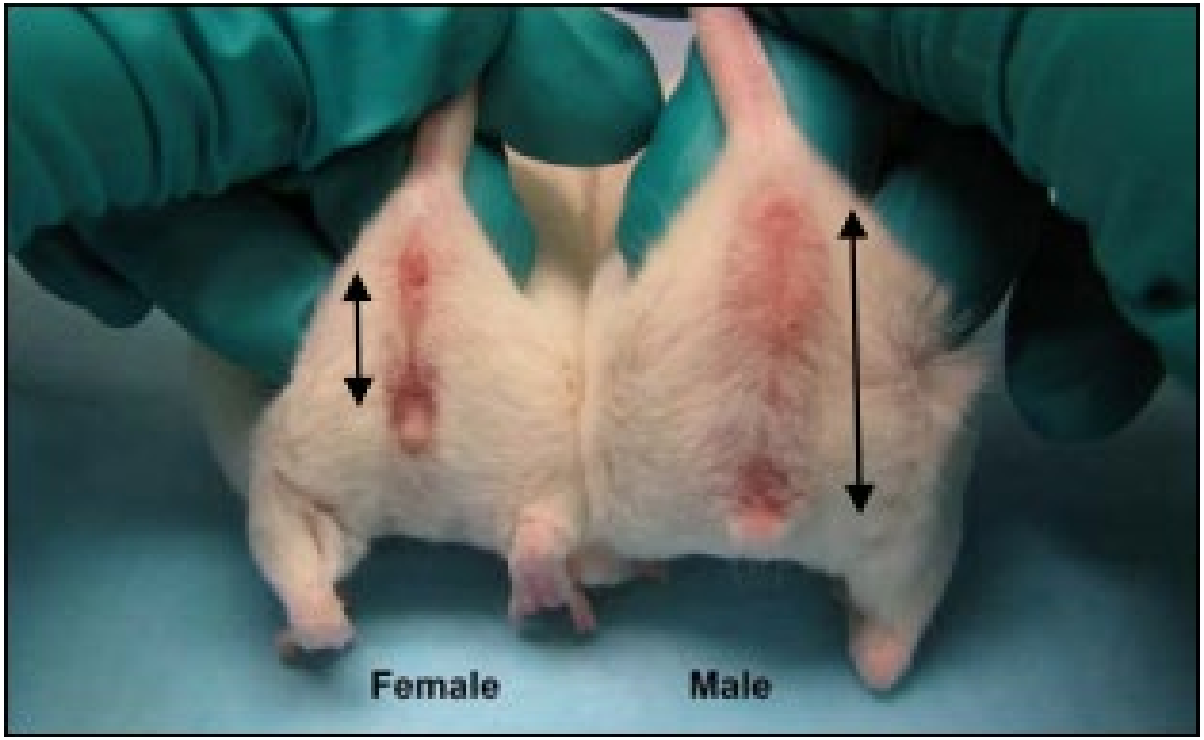
MARKING

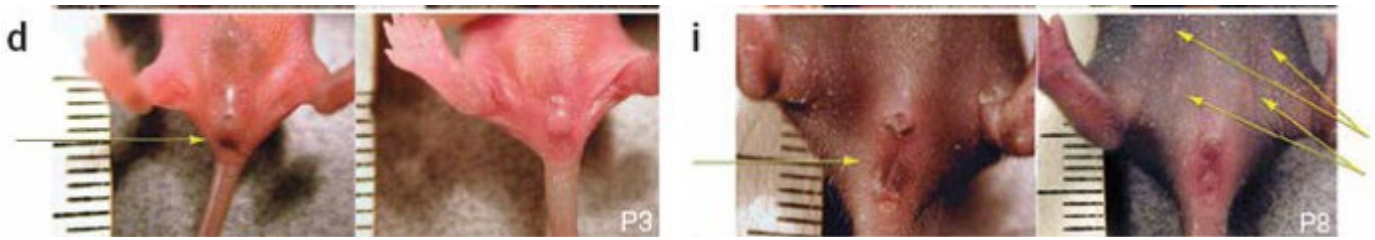
- ear punching,
- ear tagging,
- tattooing,
- and subcutaneously implanting a numerically coded microchip.





SEXING





PLUG CHECK

- For setting up timed pregnant mice.
- Foster mothers.

- [Appearance of the vagina in proestrus]

[Vaginal plug]

ROUTES OF ADMINISTRATION

Injections:

- Intraperitoneal (i.p.).
- Subcutaneous (s.c.).
- Intramuscular (i.m.).
- Intravenous (i.v.).
- Intradermal (i.d.).

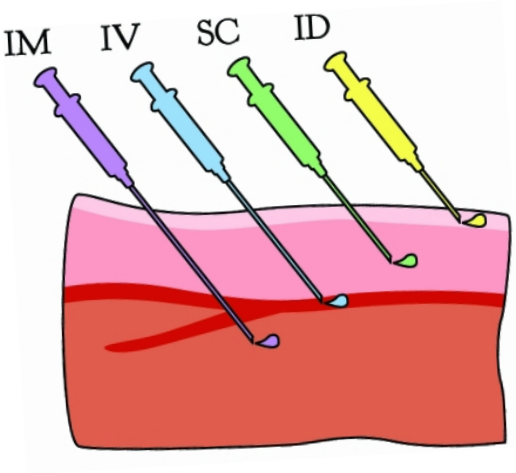


Illustration: Gianni Chiappetta

- Use a fresh, sterile needle.
- Inject with the bevel of the needle facing up.
- Do not reuse needles between animals.
- Calculate dose, ensure volume is within limits.

INJECTION SITES, VOLUMES & ROUTES

Route	Recommended site[26]	Needle gauge[26]	Maximal volume[27]
subcutaneous	dorsum, between scapula	25-26 ga	2-3 ml
intraperitoneal	left lower quadrant	25-27 ga	2-3 ml
intravenous	lateral tail vein	27-28 ga	0.2 ml
intramuscular	hindlimb, caudal thigh	26-27 ga	0.05 ml

ROUTES OF ADMINISTRATION

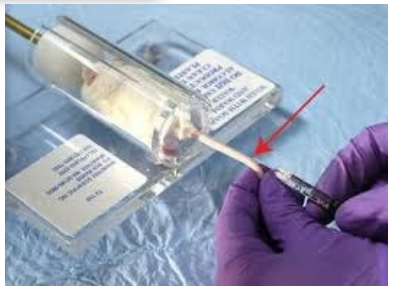
Intraperitoneal: Into the posterior quadrant of the abdomen, 45 degree angle, always aspirate!

Subcutaneous: Into the scruff of the neck, minimal pain or discomfort.

Intradermal: difficult in mice, not recommended, (0.01ml V).

Intramuscular: into the quadriceps muscle groups on the anterior of the thigh.

Intravenous: Mouse-restrainer, heat lamp to warm tail for dilation of the vein.



BLOOD COLLECTION

Blood collection not requiring anesthesia:

- Tail vein
- Mandibular vein
- Saphenous vein
- Dorsal pedal vein

Blood collection requiring anesthesia (local/general anesthesia)

- Tail snip

Terminal procedure

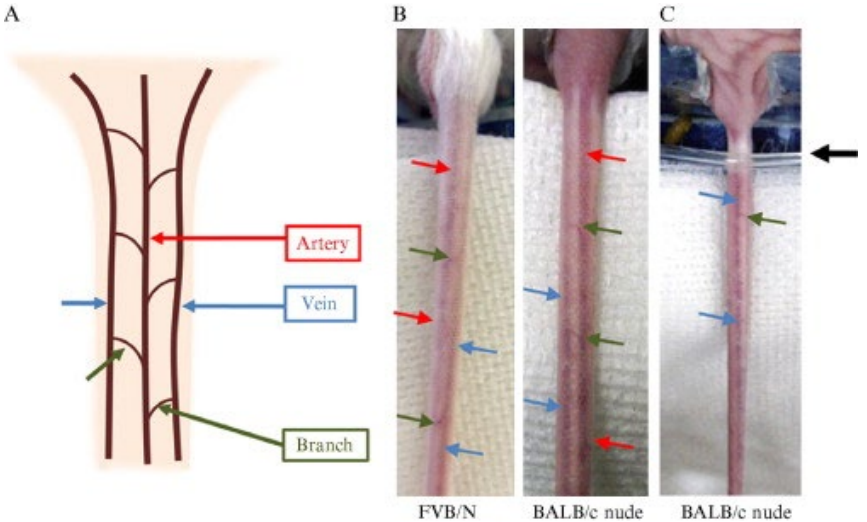
- Cardiac puncture
- Orbital sinus
- Posterior vena cava

BLOOD COLLECTION

	Mouse (25 g mouse)
Blood volume	74 mL /kg (range 70–80) 6.6% BW 1.8 mL
Safe single sample	0.2 mL (10%) max 0.1 mL (7.5%)
Continuous samples	
Day	0.02 mL
Week	0.14 mL

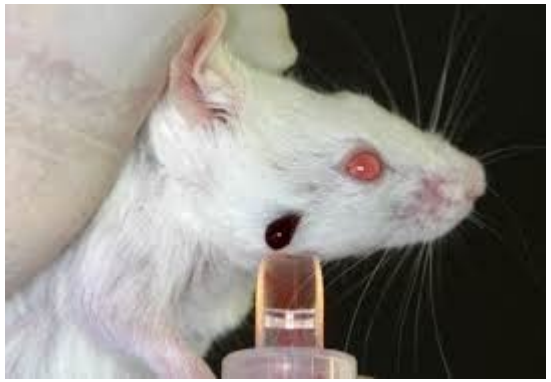
TAIL VEIN

- restrain animal
- heat the tail
- locate puncture site
- puncture with a lancet or needle
- collect blood (labeled collection tube)
- apply pressure with a gauze
- release animal and check status.



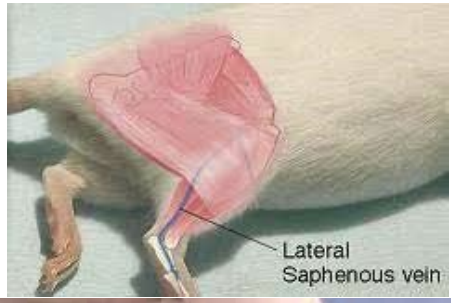
MANDIBULAR VEIN

- restrain animal
- locate puncture site
- puncture with a lancet or needle
- collect blood (labeled collection tube)
- apply pressure with a gauze
- release animal and check status.



SAPHENOUS VEIN & DORSAL PEDAL VEIN

- restrain animal
- properly shave the leg
- locate puncture site
- puncture with a lancet or needle
- collect blood (labeled collection tube)
- apply pressure with a gauze
- release animal and check status.



CARDIAC PUNCTURE

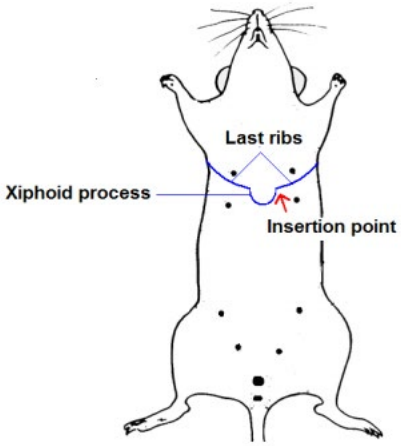
Anesthetize!!

Access to the heart:

- left lateral (closed)
- through diaphragm (closed)

  insert the needle at the base of the sternum, below the xiphoid, bevel up, into the thoracic cavity at a 15 - 20° angle directed just to the left of the midline
- thoracotomy (open)
- through diaphragm after laparotomy (open)

Euthanize –this is a terminal procedure!



ANAESTHESIA: CONSIDERATIONS

- Experimental procedures require anesthesia and/or analgesia:
  - Injectable or Inhalational
  - Necessary for immobilization, reduce stress or pain.
  - Drug administration.
  - Surgical prosedures.

- Three components of anesthesia:
  1. Analgesia (pain relief)
  2. Amnesia
  3. Immobilization

Medium anesthesia (most surgeries conducted at this level):

- Muscles are relaxed, most reflexes are absent
- Fast recovery (keep warm & safe).

ANAESTHESIA

Injectable:

- ***Avertine*** 2.5%

	20gr	22gr	24gr	26gr	28gr	30gr	32gr	34gr
17µl/ g ζβ	340µl	380µl	410µl	440µl	480µl	510µl	545 µl	580 µl
15µl/ g ζβ	300µl	330µl	360µl	370µl	420µl	450 µl	-	510 µl

- **Ketamine+xylazine**

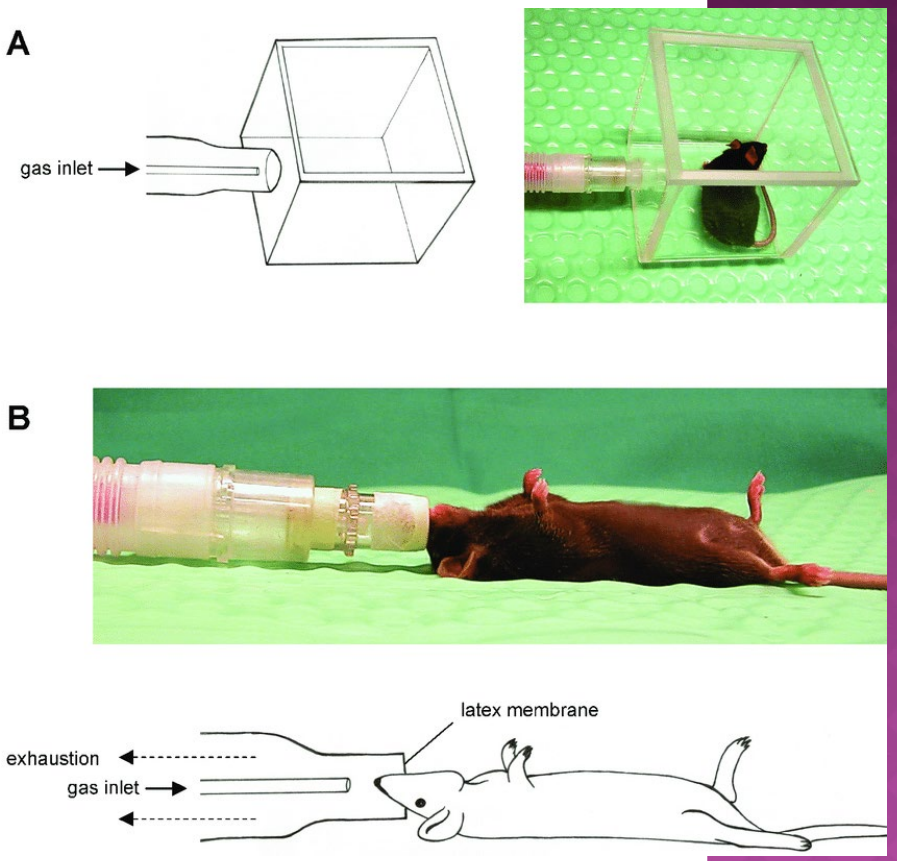
TABLE 6.5 Anaesthetic Dose Rates in the Mouse.

Drug	Dose rate	Effect	Duration of anaesthesia (minutes)	Sleep time (minutes)
Ketamine/xylazine	80–100 mg/kg + 10 mg/kg ip	Surgical anaesthesia	20–30	60–120
Ketamine/xylazine/ acepromazine	80–100 mg/kg + 10 mg/kg ip + 3 mg/kg ip	Surgical anaesthesia	30–40	60–120

- **Ketamine+dextomitor**
  - **75mg/kg + 0.5mg/kg**

ANAESTHESIA

- Inhalational **anaesthesia**:
  - Intubation or mask or box.
  - Drugs used:
    - Isoflurane
    - Sevoflurane



EUTHANASIA

- CO2 asphyxiation

Euthanasia chamber, 30-70% filling rate/minute

- Anesthetic overdose

Barbiturate (pentobarbital at 200mg/ml concentration

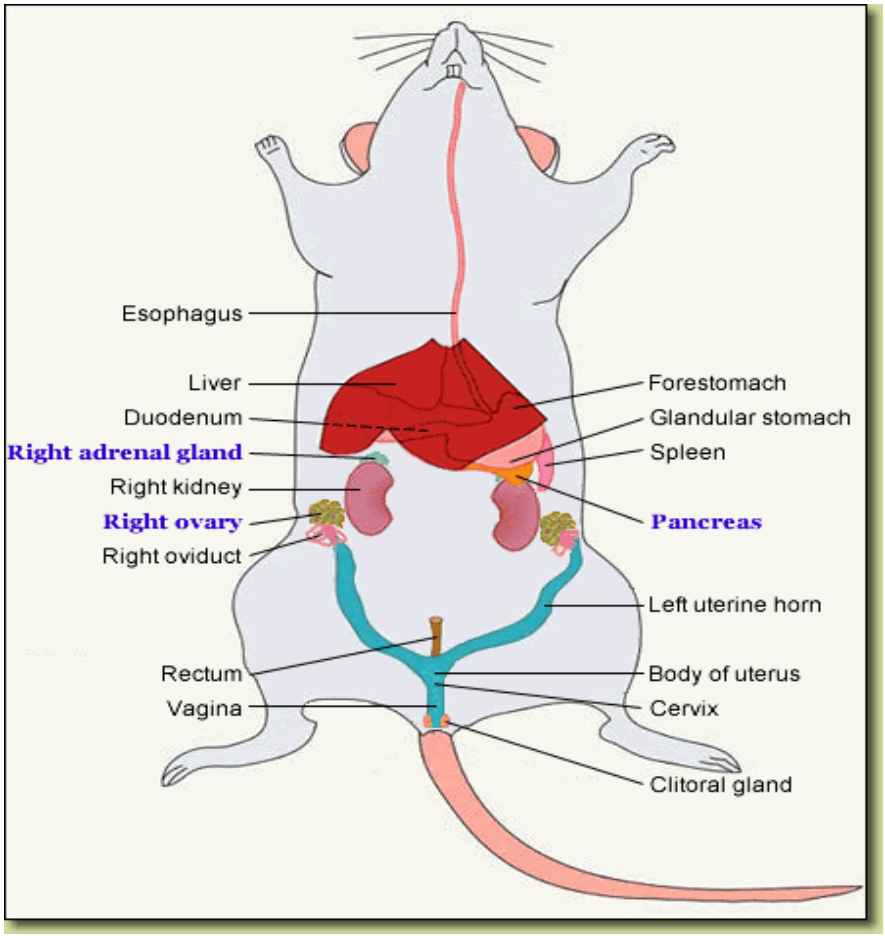
- Cervical Dislocation

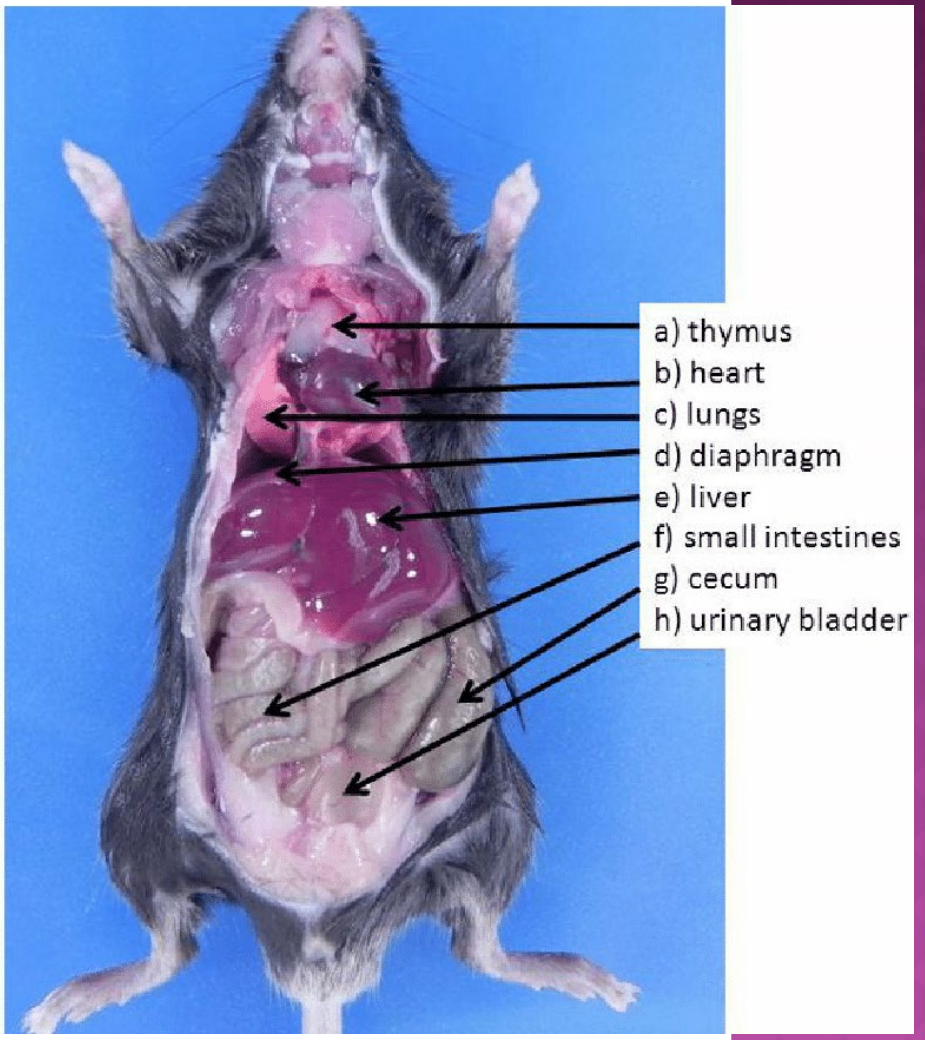
EUTHANASIA

RODENT EUTHANASIA

METHODS OF EUTHANASIA	CHEMICAL				PHYSICAL		
	CO_2 ASPHYXIATION UNDER ISOFLURANE ANESTHESIA	CO_2 ASPHYXIATION	BARBITURATE OR INJECTABLE ANESTHETIC OVERDOSE	INHALANT ANESTHETIC OVERDOSE	CERVICAL DISLOCATION	PNEUMOTHORAX	DECAPITATION
 • Adult rodent • Gestating rodent (under 17 days gestation)	YES	YES	YES	YES	YES Only after a chemical method of euthanasia or under anesthesia unless approved by the FACC	YES Only after a chemical method of euthanasia or under anesthesia	YES Only after a chemical method of euthanasia or under anesthesia unless approved by the FACC
 • Gestating rodent (over 17 days gestation)	YES*	YES*	YES	YES*	YES* Only after a chemical method of euthanasia or under anesthesia unless approved by the FACC	YES* Only after a chemical method of euthanasia or under anesthesia	YES* Only after a chemical method of euthanasia or under anesthesia unless approved by the FACC
	* Decapitation of pups required after euthanasia of the mother. If barbiturate or injectable anesthetic overdose is used to euthanize the mother, decapitation is not required.						
 • Pups less than 10 days old	Only as Narcosis Followed by another physical method of euthanasia	Only as Narcosis Followed by another physical method of euthanasia	YES	Only as Narcosis Followed by another physical method of euthanasia	NO	NO	YES

SAMPLE COLLECTION



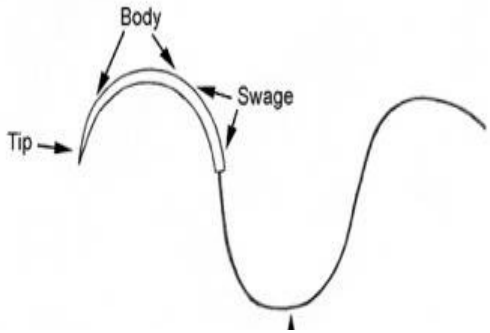


SUTURING

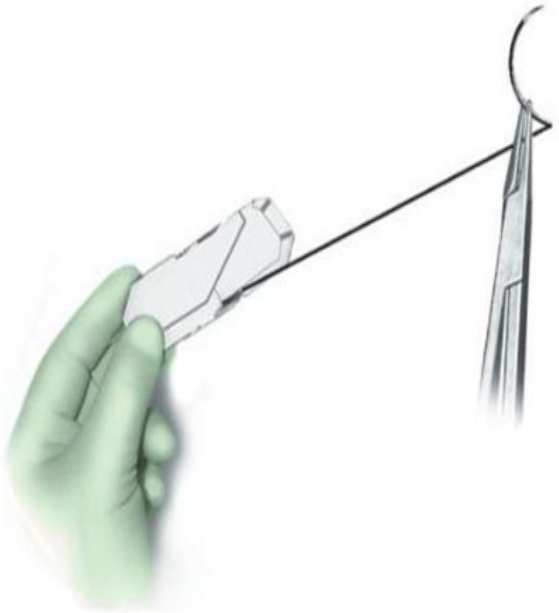
BASIC SUTURING EQUIPMENT

surgical instruments:

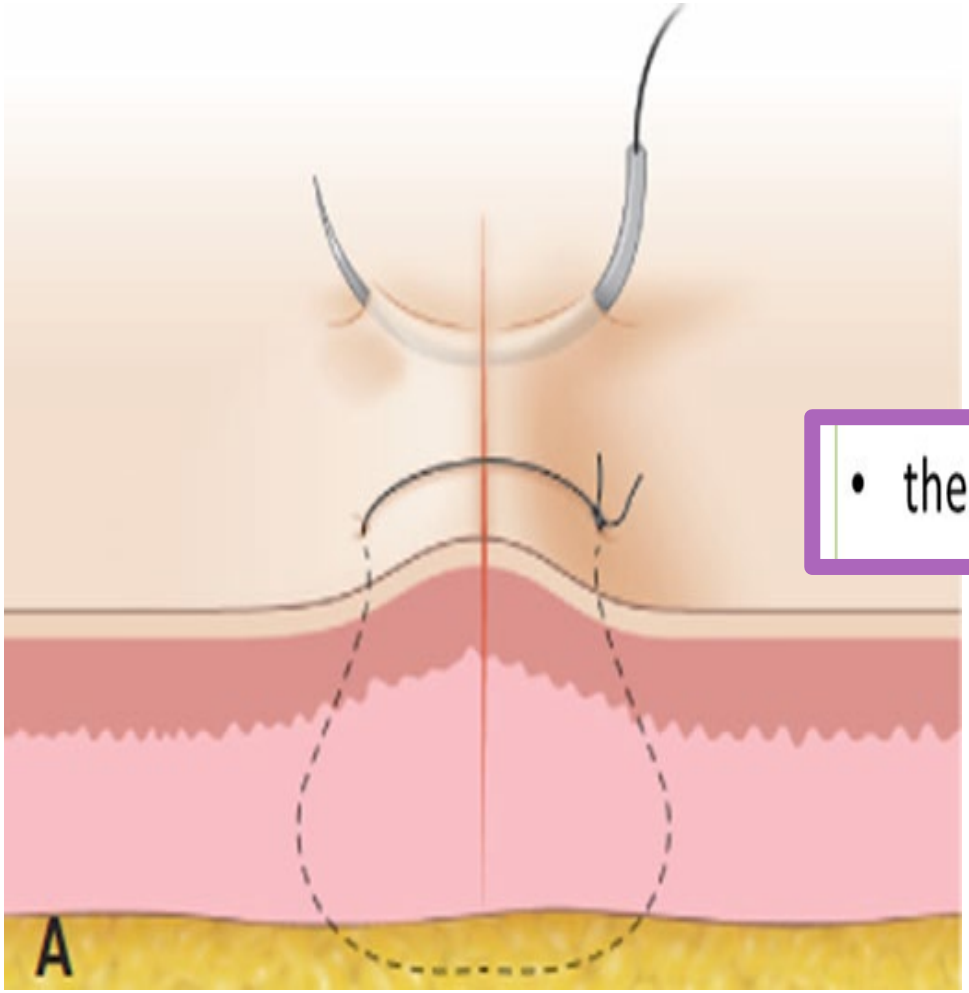
SUTURE HANDLING



Suture is removed from the package placing tension on the swage

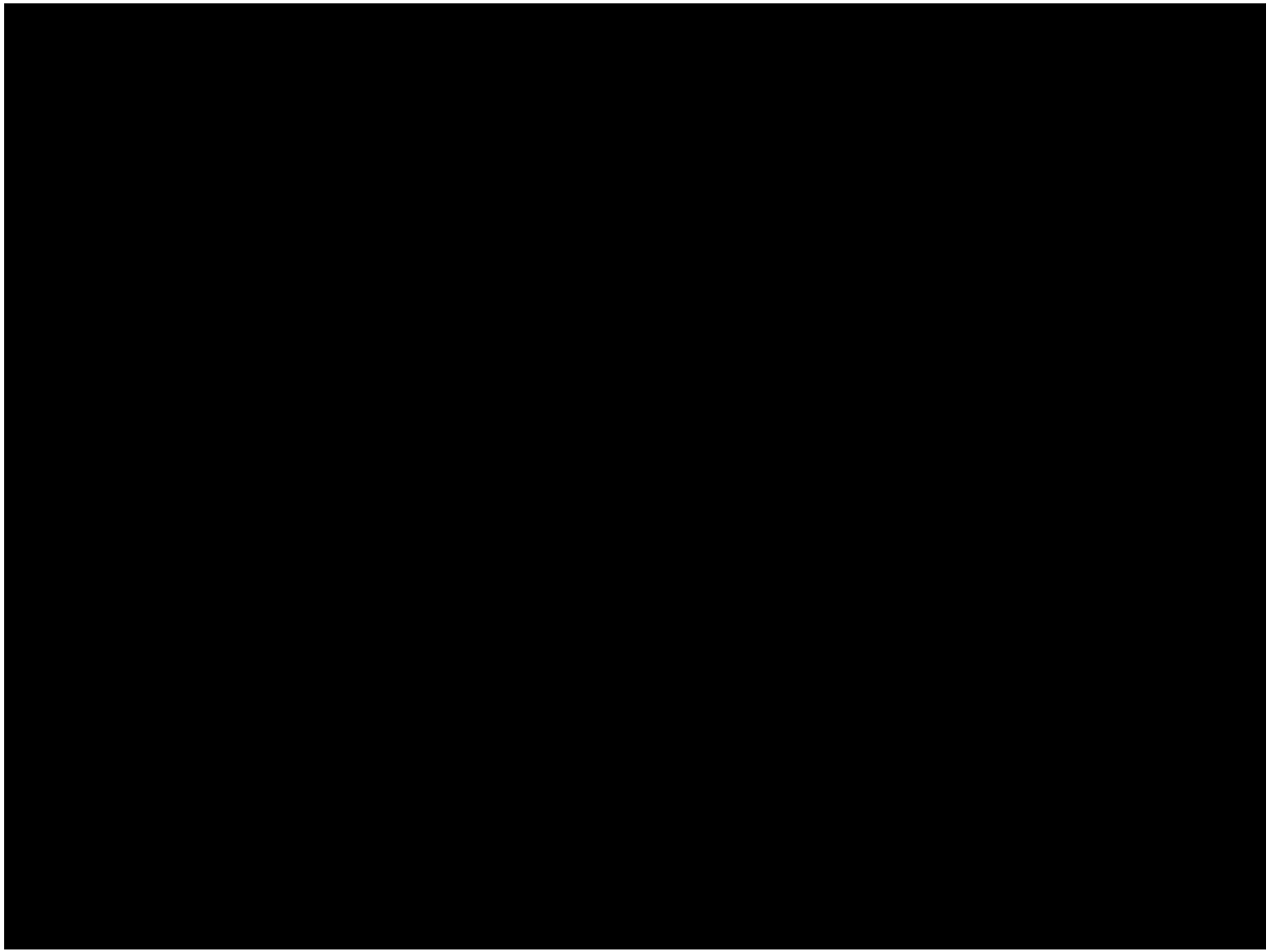


SIMPLE INTERRUPTED

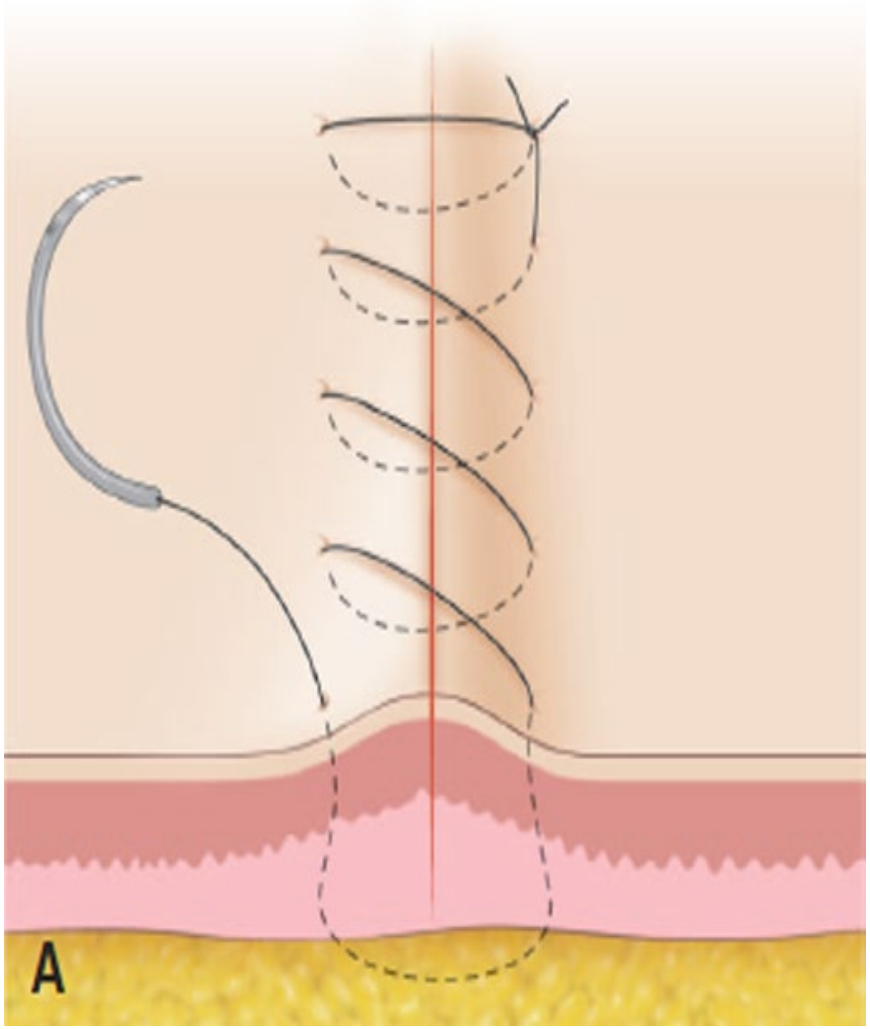


- the knot is on the side

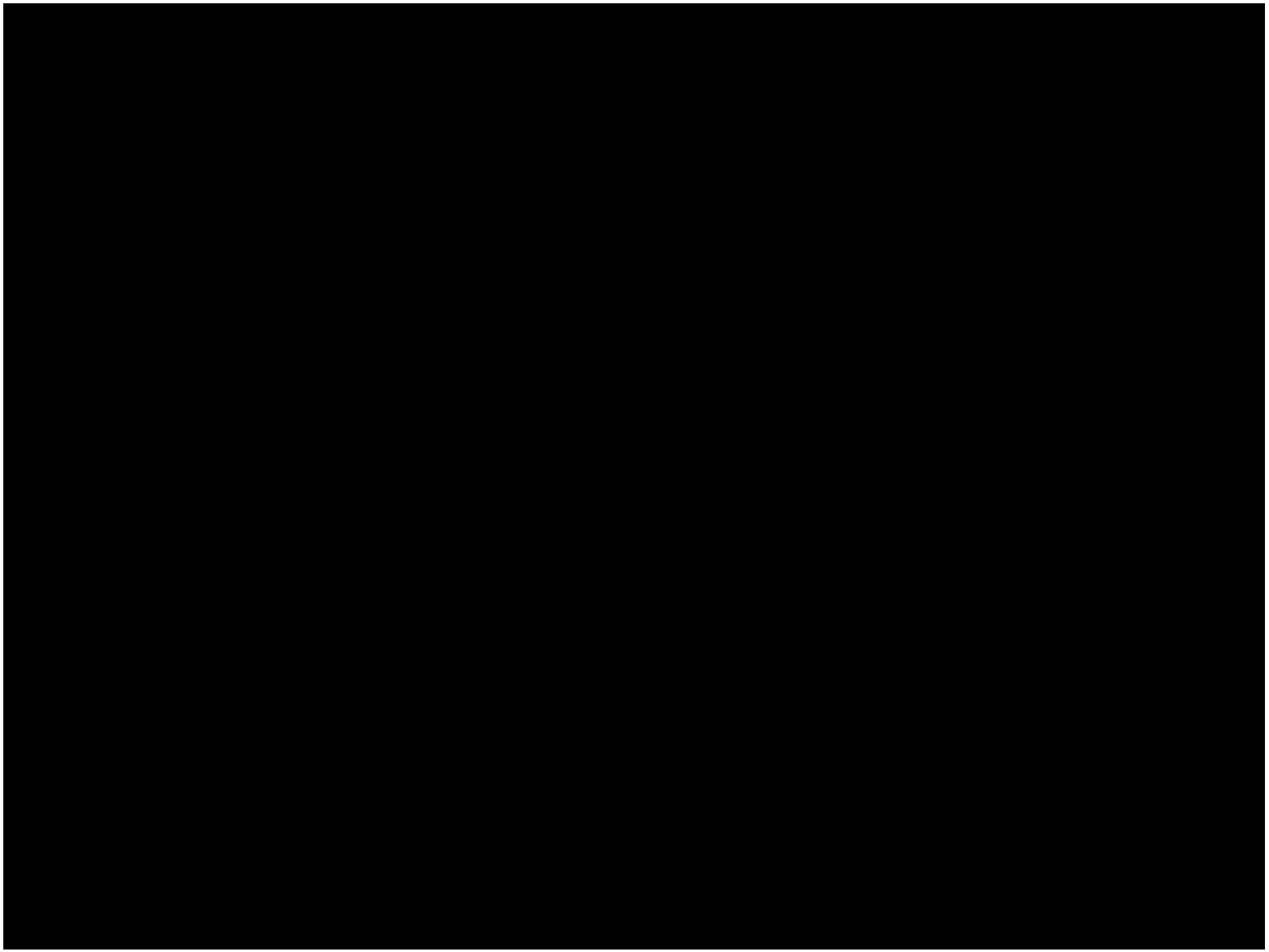
SIMPLE INTERUPTED



SIMPLE CONTINUOUS



SIMPLE CONTINUOUS



THANK YOU

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