In blood sample we may prepare

1-Serum . 2-Plasma .

1-Serum is the liquid fraction of whole blood that is collected after the blood is allowed to clot. The clot is removed by centrifugation and the resulting supernatant, designated serum, is carefully removed using a Pasteur pipette.

Serum preparation

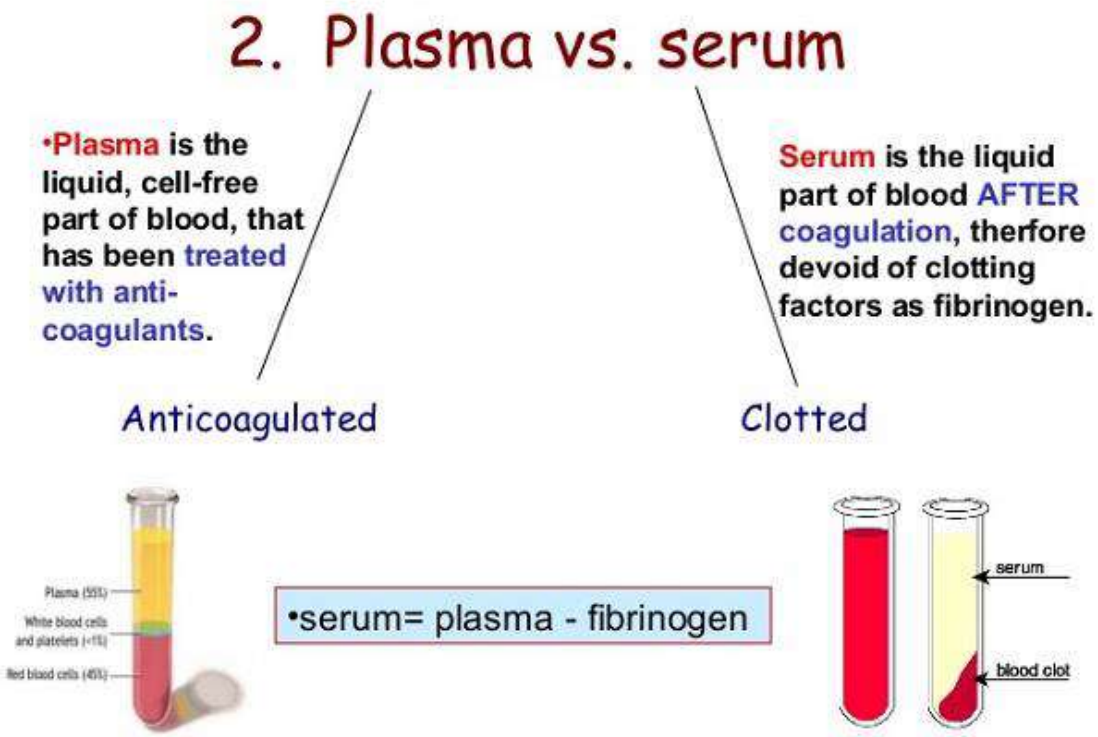
Collect whole blood in a covered test tube. After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 15–30 minutes. Remove the clot by centrifuging at 1,000–2,000 x g for 10 minutes in a refrigerated centrifuge. The resulting supernatant is designated serum. Following centrifugation, it is important to immediately transfer the liquid component (serum) into a clean polypropylene tube using a Pasteur pipette. The samples should be maintained at 2–8°C while handling. If the serum is not analyzed immediately, the serum should be stored at –20°C or lower. It is important to avoid freeze-thaw cycles because this is detrimental to many serum components.

2-Plasma is produced when whole blood is collected in tubes that are treated with an anticoagulant. The blood does not clot in the plasma tube. The cells are removed by centrifugation. The supernatant, designated plasma is carefully removed from the cell pellet using a Pasteur pipette

Plasma preparation

Collect whole blood into commercially available anticoagulant tubes e.g., EDTA tube. Cells are removed from plasma by centrifugation for 10 minutes at 1,000–2,000 x g using a refrigerated centrifuge. The resulting supernatant is designated plasma. Following centrifugation, it is important to immediately transfer the liquid

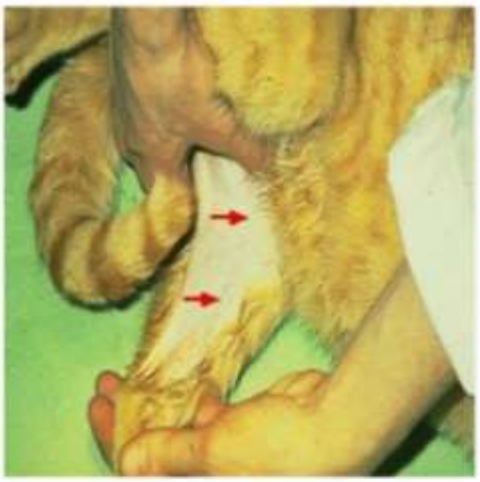
component (plasma) into a clean tube using a Pasteur pipette. The samples should be maintained at 2–8°C while handling. If the plasma is not analyzed immediately, the plasma should be stored at –20°C or lower. It is important to avoid freeze-thaw cycles.



Sites for Blood collection from different animals:

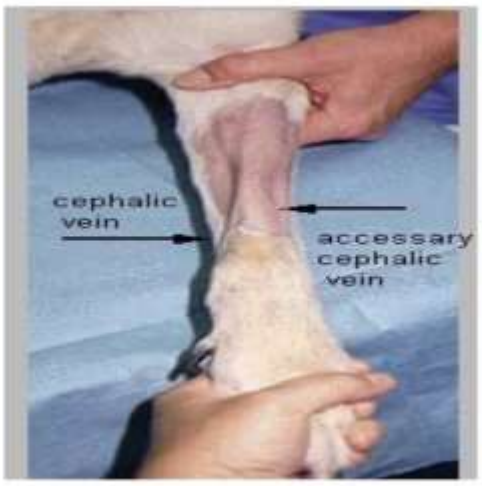
i. Collection of blood from cat

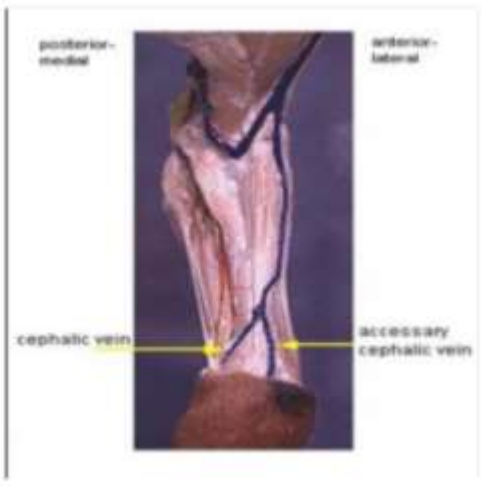
- Medial saphenous vein



ii. Collection of blood from dog

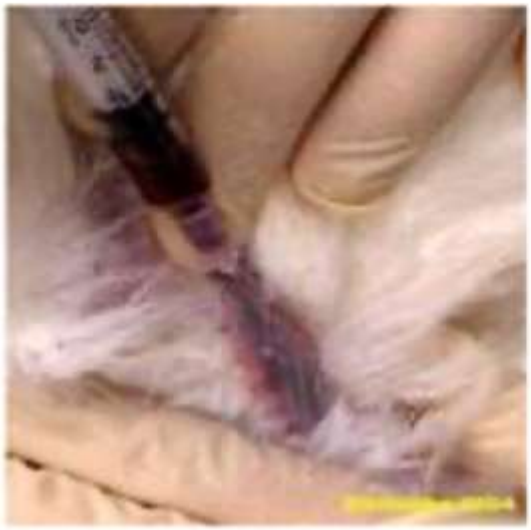
- Cephalic vein
- Jugular vein





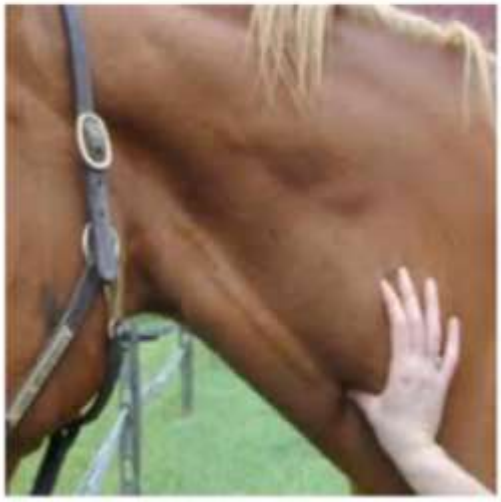
i. Collection of blood from chicken

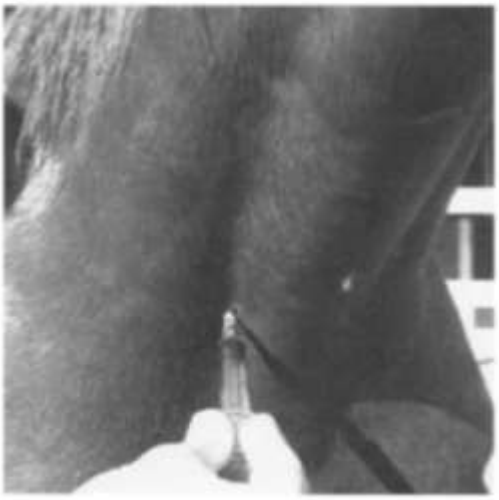
- Cutaneous ulnar or brachial veins (wing veins)
- Jugular vein of chicken



ii. Collection of blood from horse.

- Jugular venipuncture





iii. Collection of blood from sheep

- Jugular venipuncture

iv. Collection of blood from cattle

- Jugular venipuncture
- Coccygeal venipuncture

Care and handling of laboratory animals

Experimental animals which are commonly used in the laboratory are rabbits, guinea pigs, mice and poultry. Other animals may be required for special work. These animals are used for antisera production, as a source of complement, skin reactions, blood collection, anaphylactic shock and pathogenicity tests

Features uses of experimental animals

1- Rabbits: Three to four months old healthy albino rabbits weighing about 2 kg are preferred for most of the laboratory work.

2- Guinea pigs: Young adult guinea pigs about 2 to 2.5 months old and weighing about 200 to 250 gram.

3- Mice: Albino mice weighing 15 to 20 gram and about 6 to 8 weeks of age.

Handling of laboratory animals

Rabbits:

- Smooth ear of the rabbit back
- pick up the ears and loose skin at the back of the neck with one hand in a firm grip
- place the other hand under the hind quarter to support the weight and lift gently.
- Never be lifted by ear alone
- should be placed on a non-slippery surface.

Guinea pigs:

- Guinea pigs should be restrained using two hands.
- Your dominant hand should be used to grasp the animal's thorax from below opposing your thumb and fingers on either side of the animal's chest.
- The second hand is used to support the hindquarters. and then the hind limbs should be grasped and extended.

Mice:

- Place the mouse on a rough surface while holding the tail firmly.
  Note: Smooth surface will frighten the mouse because it cannot get a foothold. This may cause it to turn around and bite in its attempt to escape.
- Grasp the nape gently and firmly with your free hand and lift the mouse.
- Place the mouse's tail between the last two fingers of the hand that is holding the nape.

Rat:

- Rats may be handled by the tail, with precautions similar to those used for mice, with emphasis on only grasping the tail base.
- Holding the tail distal to the base can result in a de-gloving injury to the tail.
- Pick up the rat. Rotate the wrist of your right hand to expose the mid section.
- Extend the rat's hind legs with your left hand, grasp one hind leg between your thumb and index finger and the other between your index and second fingers.

Important notes during the injection

1. Appropriate size and sterilized syringes are required for injecting animals

2. The inoculum should be free from solid particles

3. There are no air bubbles in the syringe at the time of inoculation

4. The area of injection should be clean and sterilized with 70% alcohol.

5. The injection should be given slowly

There are various routes of injection:

1. Intradermal (ID)

2. Subcutaneous(SC)

3. Intramuscular (IM)

4. Intraperitoneal (IP)

5. Intravenous (IV)

1-Intradermal injections

Usually made on the back using small needle

Procedure

1- Clip the hairs and sterilize the area of the skin with 70% alcohol.

2- Hold the skin between the thumb and forefinger and inject 0.1ml quantity in between the two folds of the skin. A correct injection will form a pea sized white anaemic nodule at the site.

2-Subcutaneous injection

This injection is usually made on the back, abdomen, or in the groin.

Procedure

1- Clip the hairs and sterilize the area of the skin with 70% alcohol.

2- Hold the skin between the thumb and forefinger

3- Insert the needle under the skin into the loose subcutaneous tissues and inject the fluid

3-Intramuscular injection

The injection is made into the posterior muscles of the thigh

Procedure

1- Clip the hairs and sterilize the area of the skin with 70% alcohol.

2- Insert the needle into the muscle and inject the fluid

4-Intraperitoneal injection

This injection is usually made below the umbilicus in the median line

Procedure

1- Clip the hairs and sterilize the area of the skin with 70% alcohol.

2- Insert the needle the skin, penetrate the abdominal muscles and the peritoneum then inject the fluid

5-Intravenous injection

A-In rabbit this injection is made in marginal ear vein

Procedure

1. Clip the hairs and sterilize the area of the skin with 70% alcohol with slight rubbing. This will make the ear vein prominent.

2. Insert the needle in the vein directing toward the base of the ear and inject the inoculum slowly

B-In guinea pig this injection is made in superficial vein

Procedure

1. Clip the hairs on the dorsal and inner aspect of the hind leg and sterilize the part with 70%.

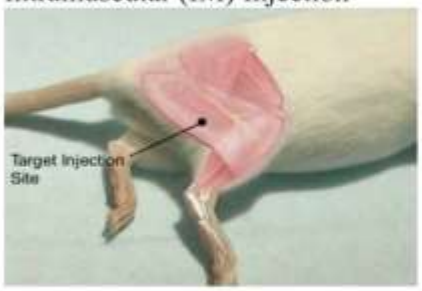
2. Insert the needle in the vein and inject the inoculum slowly

C-In mice this injection is made in tail vein

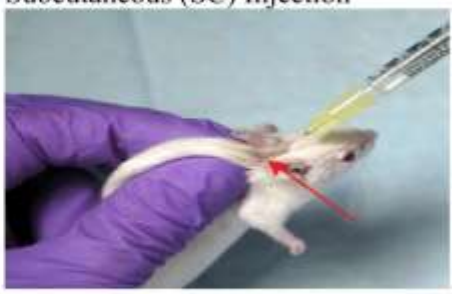
Procedure

1- Dip the ventral part of the tail with water to make the vein prominent.
2- Insert the needle in the tail vein keeping it nearly parallel to the tail with point directed towards the animal and inject the liquid.

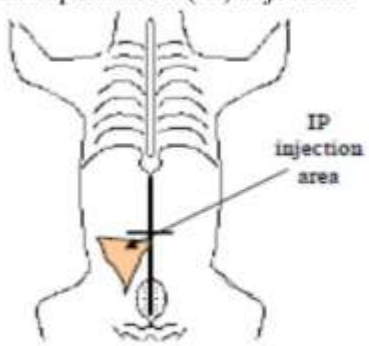
Intramuscular (IM) Injection

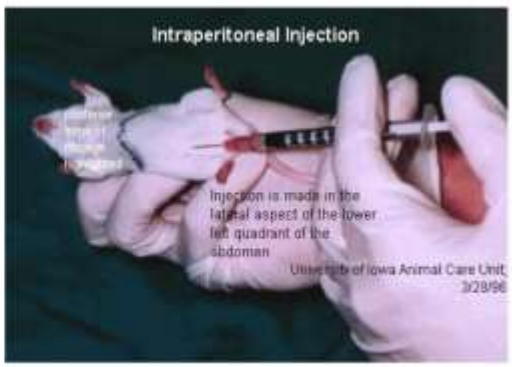


Subcutaneous (SC) Injection



Intraperitoneal (IP) Injection

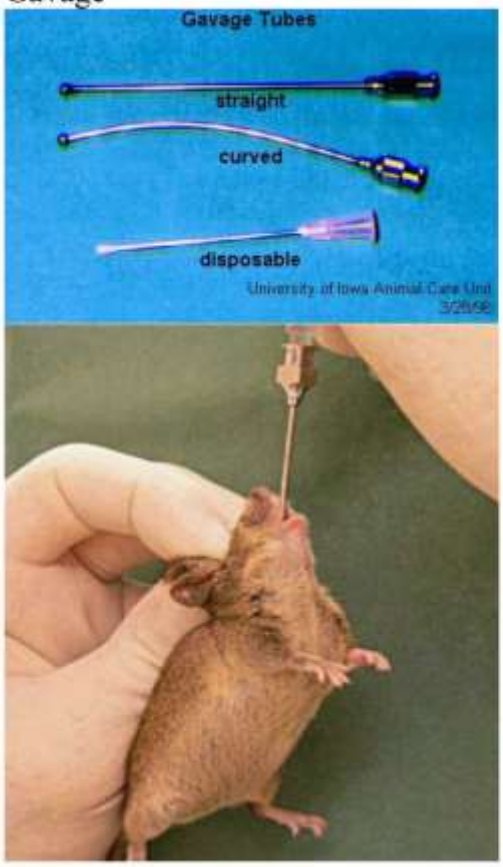


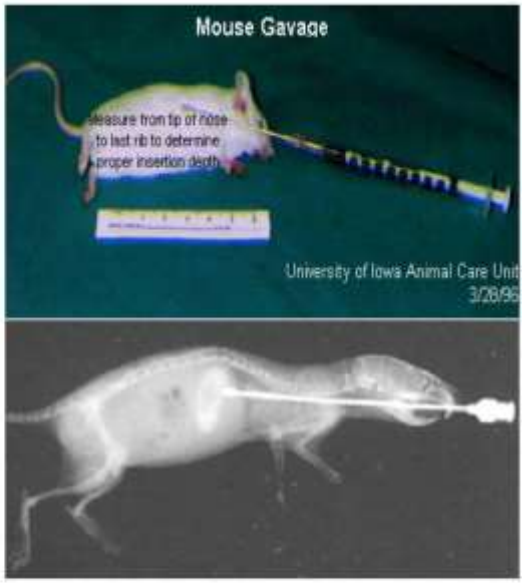


Intradermal (ID) Injection

Intravenous (IV) Injection Utilizing Lateral Tail Veins

Gavage





D-Intravenous injection in chickens is given in the wing vein.

Methods of obtaining blood from laboratory animals

Mouse & Rat

1) orbital sinus
2) lateral tail veins
3) saphenous veins
4) facial veins
5) Intra cardiac (IC) puncture

Rabbit & Guinea pig

1) marginal ear veins
2) Intracardiac (IC) puncture

Rabbits can be bled by the ear vein or from the heart

A-Bleeding from the ear vein

Procedure

1- Clip the hairs of the area over the marginal ear vein and sterilize with 70% alcohol.
2- Take a small piece of cotton dipped in xylol and rub on the tip of the ear or on the inner aspect of the ear vein. This will produce a mild inflammation making the blood vessels more prominent.

3- With the help of a sharp razor blade, make a small longitudinal slit through the skin and the vein causing the blood to ooze out drop by drop which is then collected in a wide mouth test tube.
4- When desired amount of blood has been collected press the puncture wound by a sterile cotton till bleeding stops.
B-Bleeding from the heart
1- Tie the animal securely on its back

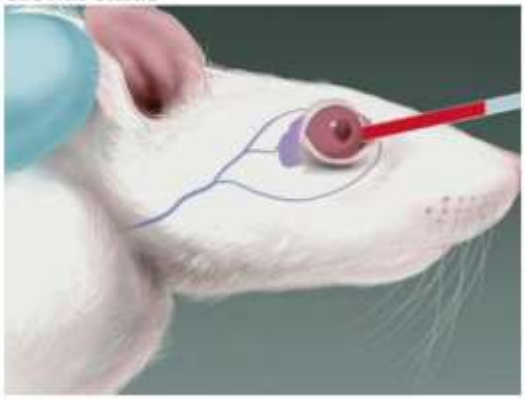
2- Clip the hair over the sternum about 1.5 inches in diameter. Sterilize the part with 70% alcohol.

3- Insert the needle through the intercostal space at area is slightly towards the left of the midway of the sternum and the needle is directed straight towards the right shoulder.

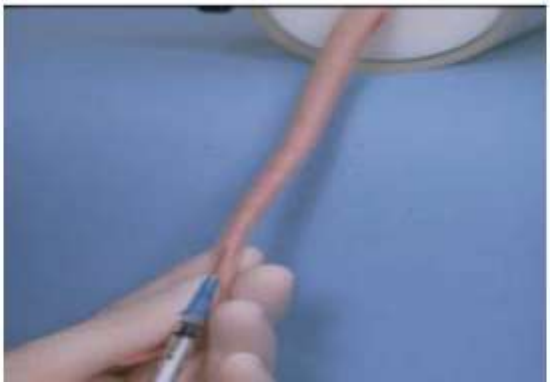
4- 30-50 ml blood can be collected without killing the rabbit.

Mouse & Rat

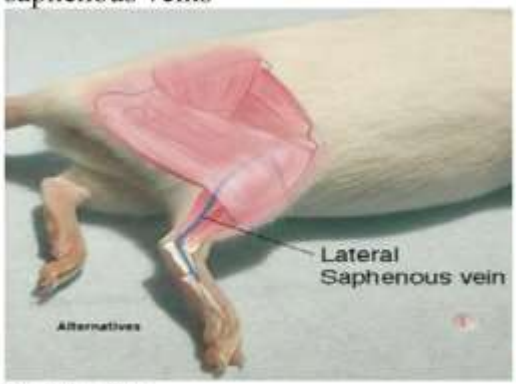
orbital sinus



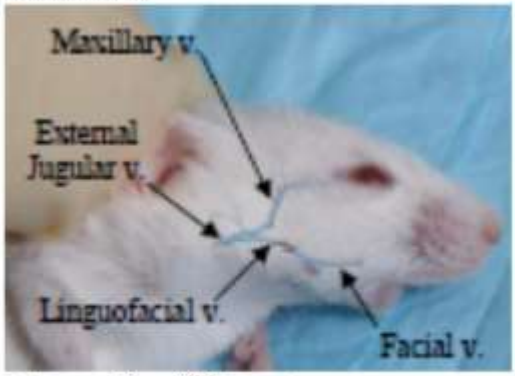
lateral tail veins



saphenous veins



facial veins



Intra cardiac (IC) puncture

Rabbit & Guinea pig

marginal ear veins

Intracardiac (IC) puncture

- Reasons of animals use for research and teaching:
  A. Biologically, humans are in the Animal Kingdom.
  B. The functions of cells and organs are basically the same in animals and humans.
  C. Animal cells function in many of the same ways as human cells.
- Uses of laboratory animals in immunology laboratory:
  - To study immune response to an antigen.
  - To produce monoclonal and polyclonal antibodies against select antigens.
  - To study hypersensitivity by producing reactions due to introduction of allergens.
  - To produce complements.
  - To use the blood / serum of animals.
  - To test and produce vaccines.
  - To study changes in the condition of animals by inoculating test substances.