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Postmortem of Laboratory Animals

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Introduction

Post- mortem procedure includes necropsy, sample collection and recording of lesions noticed. Post-mortem is majorly used for disease diagnosis, health quality control and toxicological studies. In biomedical researches, they are not only used for sample collection but also as a means for improving the reliability of experiment. These help in evaluation and characterization of mutant animals. Necropsies should be conducted following a standard protocol that should be available while conducting post-mortem and any deviation from the protocol should be noted.

In laboratory animals, infections resulting from pathogenic organisms and the lesions are uncontrolled variables that should always be investigated regularly. Diagnostic necropsies should be performed by specially trained individuals as diseases cannot be diagnosed without proper knowledge of lesions, causative factors and disease mechanisms.

Better laboratory animals will result from the systematic use of post-mortem procedures in disease diagnosis and health quality control. Defined, high quality animals and carefully designed experiments will further reduce the number of animals required.

Materials required

Surgical instruments are sufficient for most necropsies, however, certain procedures like dissection of very small organs may require microsurgery instruments. Following materials are used commonly: -

- Sharp knife/ scalpel
- Dissecting and small operating scissors
- Bone cutting forceps
- Sterile instruments for sample collection (vials, syringes, centrifuge tubes, swabs, etc.)
- Squeeze bottles containing normal saline, 10% neutral buffered formalin solution
- Weighing balance
- Electric drill along with cutting disc
- Personal protective equipment's (apron, face mask, head cap, gloves, goggles, etc.)

Description of lesions

Changes observed during necropsy should be noted and this should allow the reader to form a mental picture of the changes. The location, appearance of (intact and cut) surface and severity (size, color and distribution) along with number should be described precisely. Anatomical structures are used as landmarks. For paired organs, it should be mentioned which of them is affected.

Tissue specimen photographs are most useful aid for description, documentation and teaching purposes. For reference purposes, there should be a size marker like ruler in each photograph.

Guidelines for Necropsy of Rodents and Rabbits

Recently killed animals are considered best for conducting necropsy to avoid the effects of autolysis and putrefaction. Cadaver should be refrigerated if necropsy is delayed but never freeze the specimen as freeze- thaw cycle cause marked tissue damage. The anatomical differences between rodents and rabbit do not allow using the same general necropsy technique; the technique can be modified and all the changes should be documented.

The order of conducting necropsy depends on various factors like purpose of necropsy, samples to be collected for further study and personal preference. The tissues that autolyze rapidly should be collected and preserved as soon as possible. Vital stains are sometimes injected into the vascular system to investigate vascular permeability of different tissues and to locate very small and delicate organs like thoracic duct. Small organs such as pituitary gland, adrenals and lymph nodes may be kept directly in histological cassettes after removal and immersed in fixative to prevent tissue loss and drying.

For general necropsy, it is convenient to examine the organs in following order: -

External examination-> Skin and subcutaneous tissues-> Abdomen-> Pelvis-> Mouth-> Neck-> Thorax-> Head-> Spinal cord-> Muscles-> Joints

External examination

Recording of animal species, strain, sex, animal identity like ear tags/ tattoos and body weight is primarily done. Scoring of post-mortem changes on a scale of 1 (mild) to 5 (pronounced autolysis) is done. Observe the appearance of the skin, visible mucus membranes, hair coat and the natural body orifices for any discharge and note the respective findings on spot. Inspect the flank organs in hamsters which are male secondary sexual characteristics.

Body condition scoring is done by observing the fat and muscle masses covering the osseous protuberances, similar to live animals: -

- Emaciated- absence of fat in body depots =>1
- Undernourished =>2
- Well-nourished =>3
- Over-condition =>4
- Obese =>5

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Skin and Subcutaneous tissues

In laboratory animals (except rabbit), the cadaver is placed on its back on dissection board and pinned in position. Moisten the skin with alcohol and make a midline incision from the mandibular symphysis to the anus avoiding penis in male animals. Reflect the skin on both sides of incision and inspect the subcutaneous tissues like mandibular and cervical lymph nodes, extra-orbital lacrimal glands, salivary glands and axillary and inguinal lymph nodes.

The digestive tract in guinea pigs and autolyzed cadavers is friable and the stomach and intestines will rupture during skinning procedure, therefore, avoided if not necessary.

Abdomen and pelvis

A midline incision is made from the sternum to the pelvis and two cuts through muscles along the costal arch to open the abdominal wall. Take care to not incise the underlying viscera unintentionally. Sagittal cuts are made on each side of midline to remove the floor of pelvis and examine the abdominal and pelvic organs in-situ. Observe the appearance of serous membranes and presence of any abnormal contents such as blood, serous fluid, fibrin deposits or adhesions formed between organs and noted.

Male genital organs and Urinary bladder

Extract the testis and epididymis out of the scrotum and cut the fibrous ligament attaching the tail of epididymis to the scrotum. Cut the vas deferens and remove the testicles and epididymis for inspection. Urinary bladder, accessory sex glands, urethra and penis are brought out by cutting the ureters. Urine sample is collected from the bladder.

In rodents, the presence of urethral plug in the lumen of proximal urethra (sometime extend into bladder) is considered normal in sexually active mature males. Due to high concentration of mineral crystals, the urine of rabbit and guinea pig is normally turbid.

Female genital organs and Urinary bladder

The genital organs except ovaries are partly embedded in fat and located caudal to kidneys. Dissect the vulva and vagina free from the rectum and skin and bring the genital organs and urinary bladder out by cutting the supporting ligaments. These structures may be opened for inspection of mucus membranes.

Spleen and pancreas

Examine the spleen by removing it after cutting the omentum and ligamentum gastrolienalis along the greater curvature of stomach. Pancreas is a firm and gray coloured richly lobulated organ located in the supporting ligaments of stomach and small intestine.

Stomach and Intestine

The stomach is divided in two distinct regions in mice, hamster, rat and gerbil i.e. forestomach (thicker wall) and glandular part. This demarcation is not clear in guinea pig and no distinct areas in rabbit stomach. Gut associated lymphoid tissues are concentrated in the last thick-walled part of the

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small intestine (sacculus rotundus) and in appendix vermiform is in rabbits. The caecum in guinea pigs is thin-walled voluminous organ.

The gastrointestinal tract is removed by dissecting the anus free of its surroundings and other associated ligaments and mesentery. Stomach is gently pulled caudally and cut the esophagus and place whole GIT on dissection table. After gross examination, collect the required specimens and open it to examine the mucosa layer.

Liver, Kidneys and Adrenal glands

Examine the liver lobes superficially and deep (by making serial incisions) after removing it out by incising the supporting ligaments. Examine the gall bladder (absent in rats) and its contents. After incising the ureters, remove both kidneys along with adrenal glands and examine. Examine the cortex superficially by removing the renal capsule and cut to examine internal details. It is advised to cut the kidneys perpendicular to each other so as to distinguish them under microscope with ease.

Mouth, Neck and Thorax

Inspect the oral cavity by removing the mandible. Esophagus along with larynx and trachea are removed by cutting the muscles in ventral neck region. Remove the sternum by cutting costochondral junctions on both sides to open the thorax. Sternum sample is considered appropriate for histological examination of bone marrow.

Thymus is present in the antero-ventral part of thorax in mice, hamster and rabbits while located ventrally in guinea pigs. Rats have a smaller cervical portion also. Lower respiratory tract and heart are examined grossly and fixed for microscopic inspection.

In larger species like rabbit, open the right side by incising from sinus venosus to apex of pulmonary artery running the incision parallel to interventricular septum. Open the left side by cutting the atrial wall to the apex of aorta. Remove the blood clot and examine the endocardium. Aorta and pulmonary artery may be opened, if required.

Head and Spinal cord

Skin the neck and cranium and severe the head caudal to occipital protuberance. Scissors will be sufficient for adult mice and young animals while bone cutters/ electric drill with cutting plate is preferred for adults of other species. Cranium is opened by making two lateral cuts from medial part of both orbits to foramen magnum and one frontal cuts between medial part of both orbits. To bring the brain out, cut the calvaria, olfactory lobes and cranial nerves with dissecting scissors. Pituitary gland will appear after removal of brain lying in the sella tursica covered with a thin layer of dura mater. Scalpel blade is used as a shovel to cut the dura mater covering and lifting the pituitary gland out. In rabbits, a bony projection called dorsum sellae is present over pituitary gland covering it. Open the tympanic bullae using scissors or bone cutters and examine the middle ear.

Eye ball is taken out by severing the optic nerve and associated muscles using scalpel or scissors. Take out the lacrimal and harderian gland using scalpel. Zygomatic salivary gland is also

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located in the ventral part of orbit. Inspect the nasal cavity by cutting the cranium saggitally.

Spinal cord should be fixed in-situ as it can be damaged easily while removing from vertebral column. In adult animals, it can be removed by cutting vertebral arches alternatively on the right and left side starting from first cervical vertebra.

Muscles and joints

Thigh and sub-lumbar muscles are examined by making transverse and longitudinal cuts. Examine the major limb joints by removing the periarticular muscles and joint capsule. Samples are collected by scrapping and swabbing the articular surface of bones.

Sampling techniques

The results obtained from experimental studies and diagnostics depends on the care with which samples are collected and findings are recorded. Tests that will be run on the samples greatly decided by the necropsy record.

Sampling for Morphological Examinations

Histology

Tissues must be collected as per the experimental study or diagnostic protocol. Samples collected should be containing both the affected and apparently normal or healthy tissue. Sample tissue must be weighed prior to fixation (after removal of excess blood, fat or other irrelevant tissue. Sharp knife or scalpel should be used to avoid unnecessary stretching or compression of tissues to avoid artefacts. Sections of suitable thickness (3-5 mm) in proper amount of fixative should be collected so as to allow proper fixation.

- The volume of fixative required is atleast10 times for formalin solution and atleast 20 times for alcohol-based fixatives.
- For electron microscopy, sections of 3mm or lesser thickness are fixed using karnovsky fluid or glutaraldehyde.
- Intestines are best preserved by evacuating the contents as plunging the formalin solution in the lumen to allow quick and efficient fixation.

Parasitology

Site of sampling is of much importance as parasites have specific predilection sites. For ectoparasites, skin scrapings should be taken from multiple sites and from the edge of lesion. Gastrointestinal contents should be collected in clean, leak proof containers for endoparasite examination. Samples may be refrigerated if examined later. Blood smears (thin and thick) are examined for blood parasites. Preferably, blood smears should be prepared from fresh blood obtained without anticoagulants and fixed immediately.

Sample collection for microbiology

Sampling for cultivation

As the autolysis occurs rapidly in rodents, the time lag between death and necropsy is kept minimum. It is advised to collect specimens for bacterial/ fungal/ viral culture and molecular tests

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before the organs are handled to avoid accidental contamination. Samples should be transferred to diagnostic laboratory in leak proof labelled containers on ice pack as soon as possible.

Bacteriology

Bacterial culture is a preferred technique for isolation of bacteria. Overgrowth of contaminant bacteria can result from improper sampling technique and can result in masking of actual pathogen. Sterile moistened swabs are preferred for sampling. Tissue samples can be collected after surface sterilization. In case of hollow organs (like uterus, intestine), a segment is cut after ligation of both ends.

Respiratory and genital mucosa washings are collected for mycoplasma and bacterial culture. Impression cultures can also be done from mucosal/ serosal surfaces. Commercially available swabs containing transport media are preferred to send samples to distant laboratories.

A copy containing information regarding animal details, sending authority, detailed history, necropsy findings, samples collected with date and time of sampling and tests advised should accompany the samples when sent to diagnostic laboratory.

Mycology

Sterile saline is added to keep the specimens moist (except dermatological samples). Skin scrapings must be collected after swabbing with rubbing alcohol including both the center and periphery of lesions avoiding blood. Shaft and base of hair are collected along with skin scrapings.

Virology

Specimens should be frozen at -70° C (except blood). It is advised to collect samples soon after death as postmortem decomposition inactivates many viruses. 50% buffered glycerol is preferred as preservative for viral samples.

Serology

Considered as method of choice for detecting antibodies against protozoans, viruses, few bacteria and *Mycoplasma pulmonis*. It does not discriminate between past and present infection; a positive result only indicates that the animal has been exposed to the pathogen. It may show negative results in early stage of infection as seroconversion requires time. Preferably blood sample should be collected from live animals or recently sacrificed animals. Precaution should be taken care while collecting and processing blood sample to avoid hemolysis and separate serum as early as possible. Store the sample at -70° C if not processed at the same time.

Sampling for PCR

PCR technique is becoming essential now-a-days as it gives rapid results with high sensitivity and specificity with reliable results. The greater advantage is that it can detect infection in very early stages. Body fluids, feces and tissues are required samples and test results do not depend on the immune status of the animal. Samples collected in sterile tubes or containers should be frozen rapidly in deep fridge (-80°C).

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Sampling for Nutrient analysis and Toxicity

Examine the bedding and feed material in case of suspected nutrient deficiencies or excess or contamination by heavy metals, pesticides, herbicides, mycotoxins or other substances that might affect the biological processes. Serum and tissue samples are collected in clean leak proof containers or tubes. Plastic vials or those with rubber stoppers should be avoided as they can absorb the toxins or may contaminate the samples by leaking their own chemicals. Samples should be transported on ice packs to the diagnostic laboratory in labelled containers.

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