Revised guides for organ sampling and trimming in rats and mice – Part 1

A joint publication of the RITA* and NACAD**) groups

CHRISTINE RUEHL-FEHLERT1, BIRGIT KITTEL2, GERD MORAWIETZ3, PAUL DESLEX4, CHARLOTTE KEENAN5, CHARLES R. MAHRT6, THOMAS NOLTE7, MERVYN ROBINSON8, BARRY P. STUART9, and ULRICH DESCHL7

With 51 colored figures

Received: February 20, 2003; Revised: May 28, 2003; Accepted: May 30, 2003

Address for correspondence: GERD MORAWIETZ, Department of Information Technology and Databases, Fraunhofer Institute of Toxicology and Experimental Medicine, Nikolai-Fuchs-Str. 1, D-30625 Hannover, Germany; Fax: ++49 511 5350 155, e-mail: morawietz@item.fraunhofer.de

Key words: Trimming; RITA; NACAD; rat; mouse; standardization; guidelines; skin; mammary gland; clitoral gland; preputial gland; Zymbal’s gland; tongue; salivary gland; mandibular gland; parotid gland; sublingual gland; extraorbital lacrimal gland; lymph node; esophagus; trachea; pharynx; larynx; stomach; intestine; liver; gall bladder; pancreas.

Summary

This is the first part of a series of three articles on trimming instructions of rat and mouse protocol organs and tissues in regulatory type toxicity studies. It is based on the experience made in the European RITA and American NACAD working groups and is an extended revision of trimming guides published in 1995 (BAHNEMANN et al.). The optimum localization for tissue preparation, the sample size, the direction of sectioning and the number of sections to be prepared is described organ by organ. These descriptions are illustrated for each organ by a schematic drawing and a macro-photograph showing the plane of section as well as a low power view of the H&E stained slide demonstrating the optimum “end-product”.

This revision will improve the quality and efficiency of routine procedures and facilitate daily work in the histotechnical lab. It will promote intra- and inter-study reproducibility and comparability and thus lead to a further coherence within each study and improvement of the validity of historical control data.
Introduction

The first publication of the RITA group on the standardization of sampling and trimming procedures of organs in carcinogenicity studies was issued in 1995 (Bahnemann et al.). These guides were established based on the experience of pathologists and technicians from 20 pharmaceutical and/or chemical companies and research institutes in Europe working together in the RITA pathology data base project (Morawietz et al. 1992; Morawietz and Rittinghausen 1992; Mohr 1999; Deschl et al. 2002). The primary goal of this approach was to standardize the laboratory techniques of tissue sampling and trimming procedures in terms of defining the sites at which samples should be taken, the amount of tissue which should be trimmed, the number of sections taken and the orientation of tissues on the slide. Beside the use of standardized nomenclature and diagnostic criteria (as also published and based on an initiative of the RITA group: Mohr 1992–1997, Mohr 2001), the application of standardized histology techniques is essential when comparing historical control data derived from different studies performed at different laboratories.

The RITA paper of 1995 (Bahnemann et al.) covered only the sampling and trimming of rat tissues, but was very positively received. With the kind permission of Urban & Fischer Verlag, that version of the RITA Trimming Guides has been available free on the Internet since 1998 (http://www.item.fraunhofer.de/reni/trimming). Other publications (e.g. Bono et al. 2000) followed the basic criteria as outlined in the RITA paper.

In 1994 the North American Control Animal Database (NACAD) project was established and is operating in a way similar to RITA. In particular, the same data base structure is used, the data is stored on the same Fraunhofer ITEM data base server in Hannover, Germany, and NACAD is also based on standardized nomenclature and standardized diagnostic criteria (Keenan et al. 2002). The companies involved in the NACAD project largely adapted their tissue trimming to the RITA trimming guides.

Although the initial idea was to standardize the trimming of tissues for carcinogenicity studies, the guides have also been successfully used for short term studies. Since different national or international guidelines require the processing of different protocol organs (Leblanc 2000; Bregman et al. 2003), we attempted to include the full set in this paper, knowing that not all organs are necessary for a particular study type.

Importance of standardization

The organs which must be routinely processed in a specific type of study (e.g. sub-chronic or carcinogenicity) are defined in various guidelines, regulating the approval/registration of pharmaceutical, chemical or agrochemical compounds. However, the guidelines usually do not mention which part of an organ should be examined histopathologically. Trimming differences among groups may result in poor comparability of incidence data obtained from different groups of a study, but also in comparing incidences from different studies, particularly if derived from different laboratories. Since the probability of detecting lesions is primarily related to the amount of the tissue examined, the need for standardization becomes clear. For larger organs (like lung or liver) it is necessary to define the number of sections and the specific lobe/area sectioned (e.g. left lateral lobe, right medial lobe of the liver). The cutting direction, either as a longitudinal or a transverse section, is in particular of importance for hollow organs (like the urinary bladder, uterus) in order to provide comparable areas of tissue for examination. Other technical procedures, such as installation of fixative, decalcification, and the type of fixative used for particular organs influence the probability of detecting lesions in the final histological slide. A thorough understanding of the anatomic features (sub-sites) of all organs sampled (e.g. renal cortex and pelvis, adrenal cortex and medulla, seminiferous tubules and rete testis) is important to ensure an adequate histologic evaluation of all potential target sites in a given organ.

All these requirements were set in the frame of cost effectiveness, i.e. to gain a maximum of information with an acceptable investment of resources. It is not within the scope of this article to present sophisticated trimming procedures which may be required for specially designed mechanistic studies.

Revised and enhanced trimming guides

A number of reasons triggered the revision and enhancements of the criteria published in 1995. The main three are outlined below:

- In the last couple of years, a large number of mouse studies have been entered into the RITA and NACAD data bases, and the tissues have been histotechnically processed at the participating companies primarily following the guides established for the rat. The experience gained in the laboratories and the consideration of current literature showed that in some cases, an adaptation according to the anatomical situation in the mouse is necessary.
- The original (1995) trimming guides have been intensively discussed by the participants of the NACAD group and several modifications have been proposed, based on their practical experience. These proposals and suggestions for improvement were incorporated into the current paper, so that it now presents an international harmonization among both groups.
- The involvement of technicians in the information exchange stimulated the enhancements from a practical point of view. This resulted in the inclusion of macroscopic images of the organs and scans of the histological slides. Such histological images demonstrate how the final “product” should look, if the current guides have been applied.
Instructions and illustrations in individual organ guides

The revised and enhanced trimming guides are published in a series of three papers of which this is the first. The instructions are presented according to organ systems, organ-by-organ. In general, each organ description is valid for rat and mouse tissues but most of the gross and histopathological images are taken from the rat. If differences between the two species must be considered, they are mentioned in the text and/or in the figure legends.

For each organ the following information is usually presented:

1. **Localization**: anatomical site or part of an organ from which a sample should be taken (i.e. lobe).
2. **Number of samples**: number of organs (i.e. both for bilateral organs) or organ pieces prepared for evaluation (not necessarily identical with the number of slides/blocks).
3. **Direction**: direction (plane of section) in which an organ should be cut at trimming or microtome sectioning (see also the remarks at the end of this chapter). The proposed direction is shown in green color and optional sections (if defined) are shown in blue (see fig. 1 for an explanation of the symbols used).
4. **Sample size**: the size (area) of an organ or part of an organ which is sampled in a cassette for processing. The sample size is determined by the size of an organ. For optimal fixation, sample thickness should not exceed 3–5 mm. In general, the examined area should be as large as possible and should contain the relevant anatomical structures. The tissue can be adapted to the size of cassettes by trimming the margins off.
5. **Optional remarks** are used to present additional information, such as the instillation of fixative into the lung or the urinary bladder, optional recommended sections, placements of organs in cassettes, etc.
6. **Schematic drawings** and/or **gross photographs** are given. The plane of section is usually indicated in both images. Some of the gross photographs show the organ and trimming direction in situ. However, this is just for orientation purposes and it is recommended to remove the organ or tissue first. Trimming is performed as the next step, either on the fresh wet tissue or, in most cases, after fixation of the organ. Most of the gross photographs were taken from fresh unfixed organs. After fixation, tissue shrinkage and changes in color may lead to slight variations from the photos presented here.
7. **An image of a Hematoxylin and Eosin (H&E) stained tissue section** is shown for the recommended section level (sometimes also for optional levels). Typical structures included in this section are indicated as necessary. As a routine, 10% buffered formalin (i.e., approx. 4% formaldehyde solution) is recommended as the fixative of choice. For some of the scans, the organs were fixed with Davidson’s fluid. This is not indicated in the figure legend, since it usually does not influence the appearance of tissues at the low magnification used in the scans. If a special type of fixative is appropriate for a particular organ (e.g., eye or testis), it is mentioned in the organ manuscript.
8. **If helpful, images on histotechnical utilities** (e.g. special cassettes, tools) are included.
9. **If appropriate, further information stating the reasons** for specific sectioning levels or multiple sections, as recommended by the RITA/NACAD groups, is included.
10. **References to literature** specific to a particular organ are included where appropriate and summarized at the end of each paper.

In the descriptions the following terms are used for the determination of the trimming directions (see also fig. 2 with a schematic presentation of the related cut levels):

- **transverse**: perpendicular to the long axis of an organ or part of an organ
- **longitudinal vertical**: in the direction of the long axis of the body, an organ or part of an organ in the dorsoventral axis or parallel to it (in the text also referred to in short as “longitudinal”)
- **longitudinal horizontal**: in the direction of the long axis of the body, an organ or part of an organ, perpendicular to the dorsoventral axis (in the text also referred to in short as “horizontal”)

By defining either the “body”, the “whole organ” or a “part of an organ” (for example a liver lobe or a certain part of the brain), as a unit of reference, it is relatively simple to precisely characterize a trimming direction by using only the three above defined terms and avoiding confusion.

Fig. 1. Symbols used in the drawings and/or gross photographs to indicate the plane of section. a: cutting level parallel to the plane of the picture, b: cutting level perpendicular to the plane of the picture, c: cut level, 3-D.

Fig. 2. Schematic presentation of the plane of section. a: transverse, b: longitudinal vertical, c: longitudinal horizontal.
therefore the vast amount of anatomical terms and confusing synonyms present in literature. The schematic drawings and/or the gross images of the organs both include the trimming directions as colored lines or symbols to aid in orientation and identification of the correct sections.

Conclusion

The authors believe that this revision will assist in improving the quality of routine necropsy and trimming procedures, facilitate daily work in the histotechnical lab and advance group and study comparability. It will also contribute to a further improvement of the validity of historical control data. As in the first paper (Bahnemann et al. 1995), these revised trimming guides consider relevant scientific data for the detection of induced lesions, easy intra- and inter-study reproducibility and the relationship of cost and benefit. We hope to make these trimming guides available on the Internet similarly to the first version.

Suggested reading

Besides the references mentioned in the individual organ guides, the authors suggest the following publications, if more or general information regarding anatomy, biology, histology or trimming of rodent tissues is needed. However, if chapters of these books are of particular interest for a certain organ guide, they are included in the related references.

A detailed anatomical description of the organ systems of the rat is given by Hebel and Stromberg (1986). Boorman et al. (1990) provide valuable information on the embryology, anatomy, histology and pathology of the Fischer rat, for some organs also with trimming proposals. For the mouse, comparable information can be found in the book by Maronpot et al. (1999). Extensive information on normal anatomy, histology and physiology and their implications on toxicopathological aspects can be derived from the publications by Krinke (2000) and Haschek et al. (2002).

Acknowledgements: The authors would like to thank Mr. Theodor Laforme and Mrs. Brigitte Poschmann (Bayer AG) for the gross preparation of organs and histological slides; Mrs. Petra Hartmann and Mrs. Martina Neuenhaus (Bayer AG) for scanning the histological slides, Mr. Gerd-Peter Fehlert for his help in image processing.

References

Leblanc B: Pathology and tissue sampling protocols for rodent carcinogenicity studies; time for revision. Toxicol Pathol 2000; 28: 628–633.
1 Integumentary system

1.1 Mammary gland and Skin

Localization: Inguinal region
Number of sections: 1
Direction:
a) Transverse
b) Longitudinal vertical to the direction of the hair flow
Sample size: 1 cm × 3 cm
Remarks:
- Transverse section: includes the nipple and the lateral iliac lymph node.
- Longitudinal section: the nipple is not included if the lymph node is enclosed.
- Both sections: ensure a high amount of mammary gland tissue.

The mammary gland is a paired organ. Due to the diffuse distribution of mammary gland tissue it is of no concern whether one or both sides are in the section. The inguinal region is the recommended area for harvesting mammary gland. Sections of mammary gland should be taken with associated nipple and skin. The result of histotechnique may be improved by shaving the skin at necropsy or removing the hair with scissors at trimming. Orientation of a shaved skin specimen is possible by the nipples in female animals. In male animals, the inguinal region is also preferred to examine skin and mammary gland tissue. The section will be embedded on the cut edge so that it reveals skin, subcutis and mammary gland close to the nipple. In the longitudinal section, the hair follicles will be visible in full length.

Relevant differences between rats and mice

Rats have 6 pairs of mammary glands while mice have only 5 pairs. This difference is not of practical importance since mammary tissue is abundant in the inguinal region of both species. In females, the mammary tissue extends from the salivary gland region to the base of the tail.

Related references

1 Integumentary system

1.2 Zymbal’s gland

Localization: Adjacent to the auditory canal
Number of sections: 1
Direction: Transverse
Remarks: A preparation of the Zymbal’s gland is not advisable, instead a transverse section across the base of the decalcified skull at both ethmoidal bullae is performed.

The Zymbal’s glands are made up of several lobules of modified sebaceous glands which are located at the base of the external ear (anterio-ventral). A section through the base of the skull at the level of the external ears generally results in a section plane through one or more lobules of Zymbal’s glands tissue.

Related references
ALTMAN and GOODMAN 1979, COPELAND-HAINES and EUSTIS 1990

Fig. 1.2a. Head, ventral aspect: level of Zymbal’s gland.

Fig. 1.2b. Head, dorsal aspect after removal of skull cap and brain: Zymbal’s gland (Ma: meatus acusticus externus, P: pituitary gland, Eb: ethmoidal bullae).

Fig. 1.2c. Zymbal’s gland (Z), ethmoidal bullae (Eb).
1 Integumentary system

1.3 Clitoral/Preputial gland

Localization: Subcutaneous adipose tissue, lateral to penis/cranial to vulva
Number of sections: 1
Direction: Longitudinal horizontal

Clitoral/preputial glands are modified sebaceous glands. The whole organs are removed at necropsy and embedded in toto.

Related references
Reznik and Ward 1981a, Reznik and Ward 1981b

Fig. 1.3a. Rat: preputial glands (P), testis (T), penis (Pe).

Fig. 1.3b. Mouse: preputial gland (P), testis (T), penis (Pe).

Fig. 1.3c. Mouse: clitoral glands (C) and vulva (V).

Fig. 1.3d. Rat: clitoral glands (C), vulva (V), vena femoralis (VF), abdominal muscle (M).

Fig. 1.3e. Rat: clitoral gland (left) and preputial gland (right).

Fig. 1.3f. Mouse: preputial glands with typical dilated ducts.

Fig. 1.3g. Mouse: clitoral glands.
2 Digestive system
2.1 Tongue

Number of sections: 1
Direction: Longitudinal vertical
Optional: transverse section of mid-portion
Remarks: Tip removed if organ does not fit into the cassette

The longitudinal vertical section of the tongue covers a large part of the dorsum including the dorsal prominence. Well developed papillae are found rostral to the dorsal prominence. The section also includes the lingual lesser salivary glands and should be slightly lateral to the median sulcus.

The transverse section is recommended if blood sampling from the tongue is performed.

Related references
BROWN and HARDISTY 1990, KOCIBA and KEYES 1985

Fig. 2.1a. Tongue, longitudinal section.
Fig. 2.1b. Tongue, formalin fixed, dorsal aspect. Longitudinal (green) and transverse section (blue).
Fig. 2.1c. Tongue, longitudinal section.
Fig. 2.1d. Tongue, transverse section.
2 Digestive system
2.2 Salivary glands

Localization: Cranio-ventral throat region
Number of sections: 1
Direction: Longitudinal horizontal
Remarks: Mandibular gland through largest surface
Optional: together with mandibular lymph nodes

The three salivary glands and the mandibular lymphatic center, which consists of two or three lymph nodes are removed in one piece. At necropsy it is not necessary to prepare each organ individually. The extraorbital lacrimal gland may be removed separately or in conjunction with the other glands.

All three salivary glands should be present in the section.

Related references
HEBEL and STROMBERG 1986

Fig. 2.2a. Schematic drawing of the position of salivary glands, extraorbital lacrimal gland and mandibular lymph nodes.

Fig. 2.2b. Ventral head/throat region: mandibular, parotid and sublingual glands and mandibular lymph nodes.

Fig. 2.2c. Head, ventrolateral aspect: extraorbital lacrimal gland.

Fig. 2.2d. Salivary glands (ML: mandibular lymph nodes, PG: parotid gland, SG: sublingual gland, MG: mandibular gland).

Fig. 2.2e. Extraorbital lacrimal glands.
2 Digestive system
2.3 Pharynx and Larynx (oral study)

Localization: Middle of the larynx
Number of sections: 1
Direction: Transverse
Remarks: If necessary, decalcified for one day. Nasopharynx is included in the most caudal localization of the nasal cavity

Section contains larynx and pharynx.

See also:
Larynx (inhalation study)

Related references
BOORMAN et al. 1990a

---

2 Digestive system
2.4 Esophagus and Trachea (oral study)

Localization: Region of thyroid gland
Number of sections: 1
Direction: Transverse
Remarks: Together with trachea

This procedure is recommended if the thyroids are removed for weighing or for performing a longitudinal section of the thyroid gland.

See also:
Thyroid gland
Trachea (inhalation study)

Related references
BROWN and HARDISTY 1990

---
2 Digestive system
2.5 Stomach

Localizations:
1) From cardiac region through pyloric sphincter to the duodenum
2) Across the limiting ridge with forestomach and the fundic part of the glandular stomach
3) Optional: section through the fundus

Number of samples: 2 (3)
Direction: Longitudinal vertical
Remarks: Opened along the greater curvature, mounted and fixed

The stomach of the rat is opened along or paramedian to the greater curvature and placed on cardboard or a flat piece of styrofoam. The ingesta are removed and, if necessary, the mucosa is cleaned carefully with saline solution or fixative. The stomach is spread out and fixed with about six pins. This is a prerequisite for the macroscopic orientation and the reproducible microscopic evaluation of the gastric mucosal height or actually the measurement of the mucosa by morphometric means as it avoids folds in the mucosa.

The first section is cut from the cardiac region of the stomach across fundus and antrum and the pyloric sphincter to the duodenum. If the piece is too large for the normal cassette, the tissue can be cut in halves. The second section is taken from the forestomach through the fundic area, allowing the best evaluation of the limiting ridge between the forestomach and the glandular stomach. Optionally, a third section can be made through the fundic area, where the fundic glands are most bulky.

The fundic area is the thickest part of the mucosa and can vary under acid suppressing conditions or other drug-induced influences. ECL-cell hyperplasia and neuroendocrine tumors are found in the fundic mucosa.

Related references

Fig. 2.5a. Forestomach and glandular stomach.

Fig. 2.5b. Stomach spread out before fixation.

Fig. 2.5c. Section at location 1: Forestomach (FS), gastric fundus (GF), antrum (A), pylorus (P).

Fig. 2.5d. Section at location 2: Forestomach (FS), gastric fundus (GF).

Fig. 2.5e. Stomach: optional section at location 3 through fundus.
2 Digestive system

2.6 Intestine

Localizations:
1) Duodenum: 1 cm distal to the pyloric sphincter
2) Jejunum: central section
3) Ileum: 1 cm proximal to cecum
4) Cecum
5) Colon: central section
6) Rectum: 2 cm proximal to the anus

Number of sections: 6
Direction: Transverse
Remarks:
Duodenum in conjunction with an adjacent piece of pancreas
Jejunum containing Peyer’s patch or lymph follicle.
Optional:
additional longitudinal vertical and/or transverse section through Peyer’s patches
Cecum: due to the large diameter it is advisable to open the specimen
Rectum optional: longitudinal vertical section to include the anus

During necropsy or after fixation, the intestine is carefully separated from the mesentery. Peyer’s patches of the jejunum are mostly visible as slightly elevated lighter fields in the intestine’s wall or are even discernible as prominent areas when activated. One transverse section from each part of the unopened bowel is taken. The remaining intestine should be opened and examined for abnormalities. At necropsy, the ingesta should not be removed vigorously but only gently rinsed with physiological saline if necessary.

Swiss roll technique: This technique is sometimes required for examination of the whole intestine and the gut associated lymphatic tissue (GALT). The intestine is stripped off the mesentery, opened with a pair of scissors and gently rinsed. The intestine except cecum is recoiled on cotton swabs and fixed. After fixation, the spooled intestine is detached and embedded. This procedure is technically challenging and not recommended for routine purposes, as the intestinal mucosa and the lymph follicles will often be found cut tangential. However, transverse sections as described above will often provide a better histoanatomy.

The jejunum and ileum or the distal colon and rectum cannot readily be differentiated microscopically. For consistency in routine examination of each required site, accurate sampling is necessary. In this case, the colon differs from the rectum by a thinner muscle layer and a larger lumen. For dehydration and embedding, cassettes with a subdivision are helpful.

Please note that the magnification of the histological images is not the same for all parts of the intestine.

Fig. 2.6a. Intestine in situ with mesentery and mesenteric lymph nodes.

Fig. 2.6b. Intestine: for the collection of specimens, the mesentery is removed (explanations of numbers see fig. 2.6a).

Related references
Fig. 2.6c. Duodenum.

Fig. 2.6d. Jejunum (left) and ileum (right).

Fig. 2.6e. Cecum.

Fig. 2.6f. Colon.

Fig. 2.6g. Rectum.

Fig. 2.6h. Rectum, longitudinal section (optional).

Fig. 2.6i. Jejunum with Peyer’s patches (optional).

Fig. 2.6j. Tissue Tek Cassette, Fa. Vogel, Giessen, Germany.
2 Digestive system
2.7 Liver and Gall bladder (mouse only)

Localization:
1) Left lateral lobe
2a) Rat: right medial lobe
2b) Mouse: left and right medial lobe including gall bladder
3) Optional: caudate lobe

Number of sections: 2 (3)
Direction:
1, 2a, 3) Transverse,
2b) longitudinal-vertical

Remarks:
Sample sizes should be as large as possible but can be adapted so that all pieces fit into one cassette. For identification purposes, standardized shaping of one of the larger lobes can be performed. Sampling from other locations is also appropriate, if consistency is provided.

If major bile duct is required, the optimal section is the one through the left lateral lobe.

Relevant differences between rats and mice
Gall bladder in mice: the longitudinal section is preferred to the transverse one. In the longitudinal section, the gall bladder will be in anatomical conjunction with the liver lobes, whereas in the transverse section, the slice of the gall bladder is prone to lose its connection to the liver tissue.

Related references

Fig. 2.7b. Rat liver, visceral aspect.

Fig. 2.7c. Mouse liver, visceral aspect with gall bladder.

Fig. 2.7a. Liver, visceral aspect, indicating the cut levels for rats and mice.

Fig. 2.7d. Mouse: liver and gall bladder (G), sections 1 and 2b.
2 Digestive system
2.8 Pancreas

Localization: Left lobe
Number of sections: 1
Direction: Longitudinal horizontal
Remarks: A cut surface as large as possible

The left lobe of the pancreas represents a major part of pancreatic tissue being located close to the spleen in the greater omentum. For trimming, the whole left lobe is removed and fixed. A large part of the left lobe is taken and embedded, in order to achieve a cut surface as large as possible. The right lobe is removed together with the adjacent small intestine.

Related references
EUSTIS and BOORMAN 1997, POPESKO et al. 1992, RUBARTH 1958

Fig. 2.8a. Pancreas.

Fig. 2.8b. Pancreas.

Fig. 2.8c. Pancreas.
References for organ guides


Revised guides for organ sampling and trimming in rats and mice – Part 2
A joint publication of the RITA*) and NACAD**) groups

BIRGIT KITTEL1, CHRISTINE RUEHL-FEHLERT2, GERD MORAWIETZ3, JAN KŁAPWIJK4, MICHAEL R. ELWELL5, BARBARA LENZ6, M. GERARD O’SULLIVAN7, DANIEL R. ROTH8, and PETER F. WADSWORTH9

With 65 figures

Received: February 24, 2004; Revised: March 20, 2004; Accepted: April 16, 2004

Address for correspondence: GERD MORAWIETZ, Department of Information Technology and Databases, Fraunhofer Institute of Toxicology and Experimental Medicine, Nikolai-Fuchs-Str. 1, 30625 Hannover, Germany; Fax: ++49 511 5350 155, E-mail: morawietz@item.fraunhofer.de

Key words: Trimming; RITA; NACAD; rat; mouse; standardization; guidelines; nasal cavity; nasopharynx; paranasal sinus; larynx; trachea; bronchus; bronchiole; lung; testis; rete testis; epididymis; prostate; coagulating gland; seminal vessel; ovary; oviduct; uterus; uterine cervix; vagina; pituitary gland; thyroid gland; parathyroid gland; adrenal gland.
dardize tissue sampling and trimming, to improve the comparability of historical data obtained from different studies and different laboratories, ensure the presence of all relevant target sites for histopathological evaluation and provide technical advice for preparatory techniques during necropsy, fixation and trimming.

Brief introduction to the use of the individual organ guides

For each organ the following information is usually presented (for more details see part 1: RUEHL-FEHLERT et al. 2003):

1. **Localization:** anatomical site or part of an organ from which a sample should be taken (i.e. lobe).
2. **Number of samples:** number of organs (i.e. both for bilateral organs) or organ pieces prepared for evaluation (not necessarily identical with the number of slides/blocks).
3. **Direction:** direction (plane of section) in which an organ should be cut at trimming or microtome sectioning. The proposed direction is shown in green color and optional sections (if defined) are shown in blue (see fig. 1 for an explanation of the symbols used).
4. **Sample size:** the size (area) of an organ or part of an organ which is sampled in a cassette for processing. The sample size is determined by the size of an organ or the cassette. For optimal fixation, sample thickness should not exceed 3–5 mm. In general, the examined area should be as large as possible and should contain the relevant anatomical structures. The tissue can be adapted to the size of cassettes by trimming the margins off.
5. **Optional remarks** are used to present additional information, as recommended by the RITA/NACAD groups, such as the instillation of fixative into the lung or the urinary bladder, reasons for optional recommended sections, placements of organs in cassettes, etc.
6. **Schematic drawings** and/or **gross photographs** are shown indicating the plane of section. Some of the gross photographs show the organ and trimming direction in situ. However, this is just for orientation purposes and it is recommended to remove the organ or tissue first. Trimming is performed as the next step, either on the fresh wet tissue or, in most cases, after fixation of the organ. Most of the gross photographs were taken from fresh unfixed organs; shape and color may be slightly different after fixation.

7. An image of a *Hematoxylin and Eosin* (H&E) stained slide is shown for the recommended section level (sometimes also for optional levels). Typical structures included in this section are indicated as necessary. If not otherwise specified, 10% buffered formalin is recommended as the **fixative**.

In the descriptions the following terms are used for the determination of the trimming directions (see also fig. 2 with a schematic presentation of the related cut levels):

- **transverse:** perpendicular to the long axis of an organ or part of an organ
- **longitudinal vertical:** in the direction of the long axis of the body, an organ or part of an organ in the dorsoventral axis or parallel to it (in the text also referred to in short as “longitudinal”)
- **longitudinal horizontal:** in the direction of the long axis of the body, an organ or part of an organ, perpendicular to the dorsoventral axis (in the text also referred to in short as “horizontal”)

By defining either the “body”, the “whole organ” or a “part of an organ” (for example a liver lobe or a certain part of the brain), as a unit of reference, it is relatively simple to precisely characterize a trimming direction by using only the three above defined terms and avoiding therefore the vast amount of anatomical terms and confusing synonyms present in literature.

**Acknowledgements:** The authors would like to thank Mr. Theodor Laforme and Mrs. BRIGITTE POSCHMANN (Bayer HealthCare AG) for the gross preparation of organs and histological slides; Mrs. PETRA HARTMANN, Mrs. MEIKE PETERSEN and Mrs. MARTINA NEUENHAUS (Bayer HealthCare AG) for scanning the histological slides, Dr. GERPETER FEHLERT for his help in image processing; Mr. PETER KOCH and Mr. MICRO JAHNKE (BASF AG) for the photo documentation on prostate preparation; Dr. MARTIN ROSENBURCH (Bayer HealthCare AG), Dr. SUSANNE RITTINGHAUSEN and Dr. HEINRICH ERNST (Fraunhofer ITEM) for their scientific advice for organ preparation in inhalation studies and Dr. PAUL DESLEX (Pfizer Centre Recherche) for providing additional photographs and slides.

**Fig. 1.** Symbols used in the drawings and/or gross photographs to indicate the plane of section. **a:** cutting level parallel to the plane of the picture, **b:** cutting level perpendicular to the plane of the picture, **c:** cut level, 3-D.

**Fig. 2.** Schematic presentation of the plane of section. **a:** transverse, **b:** longitudinal vertical, **c:** longitudinal horizontal.
3 Respiratory system

3.1 Nasal cavity, Nasopharynx and Paranasal sinus

Localizations: 1) Posterior part of upper incisors
2) Incisive papilla
3) Second palatine crest
4) First molar teeth

Number of sections: 1 (oral toxicity study: third level)
4 (inhalation study)

Direction: Transverse
Remarks: Embedded with the rostral faces down
Decalcified

The structures of the palate and the teeth are used for orientation to achieve transverse sections through the nasal cavity at certain levels.

In inhalation studies, four transverse tissue levels should be taken, because the examination of these sections at defined levels assures consistent recognition of degenerative and proliferative lesions of all different epithelial cell types of the nasal cavity and paranasal sinus. Typically in oral toxicity studies, only the third level is examined. Neoplastic lesions occur more frequently in the anterior and middle portions of the nasal cavity, whereas some non-neoplastic and neoplastic lesions are observed exclusively in the olfactory epithelium. The third level includes respiratory and olfactory epithelial cells. The resulting slices of tissue are embedded with the rostral face down, because non-neoplastic lesions have been found to be most severe at the more rostral borders of the affected epithelium. Slight differences of the cut level may occur depending on anatomical variations in different strains.

If more squamous epithelium is required for examination, a section rostral to level 1 should be performed. For examination of the olfactory bulb, a section caudal to level 4 is recommended.

**Relevant differences between rats and mice**

Mice should be trimmed in the same manner as rats. However, in inhalation studies with very young or very small mice (e.g., transgenic strains), it can be difficult to cut the nose in four levels. For those exceptions, the following three level-procedure is recommended:
1) Immediately posterior to the incisors,
2) At the level of the incisive papilla,
3) Through the middle of the second molar tooth.

For non-inhalation studies level 3 is recommended for examination.

**Related references**


Fig. 3.1a. Nasal cavity, rat, 4 trimming locations.

Fig. 3.1b. Nasal cavity, rat, 4 trimming locations.

Fig. 3.1c. Nasal cavity, rat, location 1.

Fig. 3.1d. Nasal cavity, rat, location 2. Nd: nasopalatine (incisive) duct, Nl: Nasolacrimal duct.
**Fig. 3.1e.** Nasal cavity, rat, location 3. Ps: paranasal sinus.

**Fig. 3.1f.** Nasal cavity, rat, location 4. Pd: pharyngeal duct (nasopharynx).

**Fig. 3.1g.** Nasal cavity, mouse, 3 trimming locations.

**Fig. 3.1h.** Nasal cavity, mouse, location 1.

**Fig. 3.1i.** Nasal cavity, mouse, location 2. Ps: paranasal sinus.

**Fig. 3.1j.** Nasal cavity, mouse, location 3.
3 Respiratory system
3.2 Larynx

Localizations:
1) Base of epiglottis
2) Ventral pouch
3) Cricoid cartilage (rats only)

Number of sections:
Inhalation studies: rats 3, mouse 2
Optional for rats and mice: if necessary, the larynx can also be embedded in one block and step sections are taken at the predilection sites.

Oral studies: 1

Direction: Transverse

Remarks:
Since the larynx of mice is very small, only two pieces (level 1 and 2) are trimmed.

For oral studies the organ is cut at level 2 to include the most sensitive parts: ventral pouch and vocal processes (medial surfaces) of arytenoid cartilages.

Only the cranial portion of the epiglottis is removed to ensure inclusion of the major predilection site for induced lesions. This site is primarily represented by the epithelial lining of the ventral and ventrolateral luminal surface of the larynx (cranial to the ventral laryngeal pouch). The remaining larynx is trimmed according to the proposed scheme at three levels including base of epiglottis, ventral diverticulum and cricoid cartilage. The three pieces are embedded with the cranial cut surface downwards. The three levels assure recognition of all different epithelial cell types of the larynx and underlying seromucinous glands.

See also:
Thyroid gland

Related references

**Fig. 3.2a.** Larynx, inhalation studies. Rats: levels 1–3, mice: levels 1 and 2.

**Fig. 3.2b.** Larynx, level 1. Sg: seromucinous glands at the base of the epiglottis.

**Fig. 3.2c.** Larynx, level 2. Vp: ventral pouch, A: processes of the arytenoid cartilages, U: u-shaped cartilage.

**Fig. 3.2d.** Larynx, level 3. C: cricoid cartilage.
3 Respiratory system
3.3 Trachea (inhalation study)

Localization: Including the bifurcation
Number of sections: 1 (2)
Direction: Longitudinal horizontal
Optional: transverse
Remarks: Embedded in toto; careful microtome sectioning until recommended cutting level is obtained.

In inhalation studies, tracheal epithelium including the epithelial lining of the bifurcation should be examined, because this is known as the most sensitive area to respond to inhaled particulate irritants. For this purpose, a longitudinal horizontal section should provide a long distance of the epithelial surface and the tip of the carina. For optimal estimation of e.g. mild hyperplasia, it can be helpful to have an optional transverse section (see alternative trimming technique for oral toxicity study under thyroid gland/trachea/parathyroid gland).

Related references
GOPINATH et al. 1987, SCHWARTZ et al. 1991

Fig. 3.3a. Trachea with bifurcation.

Fig. 3.3b. Trachea with bifurcation, C: carina.
3 Respiratory system
3.4 Lung

Oral study: Rats and mice

Localizations: Recommended procedure:
1) Left lobe
2) Optional: right caudal lobe
3) Optional: right cranial lobe

Number of sections: 1 (3)
Direction: Longitudinal horizontal
Optional: transverse
Remarks: Instillation strongly recommended. Sectioning to the axis of the lobar bronchus. Longitudinal section comprising the lobar bronchus and its main branches. Sample size(s) adapted to the size of the cassette(s).
Alternative procedure:
Rat: right lobes embedded ventral surface down.
Mouse: whole lung embedded, ventral surface down.

Inhalation study: Rats

Localizations: 1) Left lobe
2) Right caudal lobe
3) Right cranial lobe
4) Right middle lobe
5) Accessory lobe

Number of sections: 5
Direction: Sections 1, 2: longitudinal horizontal
Sections 3, 5: transverse
Section 4: longitudinal vertical
Remarks: Instillation obligatory. Longitudinal horizontal section comprising the lobar bronchus and its main branches. Sample size(s) adapted to the size of the cassette(s); preferentially, the diaphragmatic margin is trimmed off.
Alternative procedure: right and left lobes (separate blocks) embedded ventral surface down.

Inhalation study: Mice

Localizations: 1) Left lobe
2) Right caudal lobe
3) Right cranial lobe
4) Right middle lobe
5) Accessory lobe

Number of sections: 5
Direction: Sections 1, 2, 4, 5: longitudinal horizontal
Section 3: transverse
Remarks: Instillation obligatory. Similar procedure as in rats, but lobes are embedded in toto, ventral surface down and detached from the trachea. The five lobes normally fit into one cassette.
Optional: whole lung in toto (ventral surface down) without removal of the trachea.
Microtome sectioning of left lobe and right caudal lobe until lobar bronchus and its main branches are visible (longitudinal-horizontal axis).

Spontaneous neoplastic pulmonary lesions are rare in rats and arise mostly in the lung periphery whereas regenerative hyperplasia and squamous metaplasia occur mainly in the centroacinar region. Therefore tissue of the lung including parenchyma, bronchiolo-alveolar junctions and main bronchi should be investigated. In oral toxicity studies, at minimum, one longitudinal section of the left lobe should be examined. Additionally, transverse sections of the right cranial and caudal lobes may be examined. In these sections, the epithelium of the major bronchioles, which is one important site of lesions,
can be examined at its widest diameter. In inhalation studies, sections of all five lobes should be examined according to the proposed scheme, which facilitates unambiguous identification of individual lung lobes. For histological identification of proliferative lesions in the lung, careful fixation by intratracheal instillation is recommended, even for oral studies.

**Fig. 3.4c.** Lung, rat, ventral aspect, inhalation study.

**Fig. 3.4d.** Lung, rat, ventral aspect, inhalation study.

**Fig. 3.4e.** Lung, mouse, in toto (option).

**Fig. 3.4f.** Lung, rat, location 1, left lobe.

**Fig. 3.4g.** Lung, rat, location 2, right caudal lobe.

**Fig. 3.4h.** Lung, rat, location 3, right cranial lobe.

**Fig. 3.4i.** Lung, rat, location 4, right middle lobe.

**Fig. 3.4j.** Lung, rat location 5, accessory lobe.

**Related references**
4 Male genital system
4.1 Testis and Rete testis

Localization: Transverse, close to the rete testis
Number of sections: 2 (1 per side)
Direction: Transverse
Remarks: A transverse section containing the area of the rete testis provides good histology of seminiferous tubules as well as of the rete testis. Optional: longitudinal vertical section containing the rete testis.

Near the capsule, focal tubules with flat or incomplete epithelium can be found. These should not be confused with atrophic or degenerating tubules, as they represent tubules of the rete testis. Generally, the rete testis is very small in rodents and not well visible in the histological section. In mice it is slightly more prominent than in rats and hyperplasia of the rete testis can be observed in older animals. For inclusion of the rete testis in the histological section orientation is given by the vasculature.

In short-term studies, fixation with Davidson’s or Bouin’s solutions is highly recommended to detect less extensive toxicity.

Leydig cells (interstitial cells) are present in small groups in the interstitium between the seminiferous tubules. They are found in a similar distribution in all sections proposed.

Related references

Fig. 4.1a. Testis, recommended transverse section (green), optional section (blue).
Fig. 4.1b. Testis, transverse section left, optional longitudinal section right.
Fig. 4.1c. Rete testis (R), rat (V: vasculature).
Fig. 4.1d. Rete testis (R), mouse (V: vasculature, L: Leydig cells).
4 Male genital system

4.2 Epididymis

Number of sections: 2 (1 per side)
Direction: Longitudinal vertical
Sample size: Whole organ

It appears that the epithelium in the body of the organ is sometimes more sensitive than in the head and tail. Therefore, the body should not be excluded from the investigation. The whole organ should be fixed and embedded. It should be noted that some toxicants affect in particular the efferent ducts, which are located between testis and epididymal head. Care should be taken that these structures are not destroyed during preparation.

Related references

4 Male genital system

4.3 Seminal vesicle and Coagulating gland

Localization: In the mid portion
Number of sections: 2 (1 per side)
Direction: Transverse
Remarks: Together with coagulating gland

The coagulation gland represents the dorsocranial part of the prostate.
A transverse section should be made through the widest part of seminal vesicle together with coagulating gland.

Related references
BOORMAN et al. 1990b, CREASY and FOSTER 2002, FERM 1987, SUWA et al. 2002

Fig. 4.2a. Epididymis.

Fig. 4.2b. Epididymis (B: body, H: head, T: tail).

Fig. 4.2c. Epididymis (B: body, H: head, T: tail).

Fig. 4.3a. Seminal vesicle and coagulating gland.

Fig. 4.3b. Seminal vesicle (Sv) and coagulating gland (Cg).
4 Male genital system
4.4 Prostate

Localization: Dorsolateral and ventral lobe
Number of sections: 1
Direction: Longitudinal horizontal after special preparation (see below).

Fig. 4.4a. Prostate, rat, ventral aspect. Lateral lobes not visible, dorsal lobe: only caudal part visible.

Fig. 4.4b. Prostate, rat, dorsal aspect.

The dorsolateral and ventral lobes that normally lie in a vertical axis above each other (with urinary bladder and seminal vesicles in between) are spread in a horizontal axis and embedded with the “outer” aspect down into the cassette.

Preparation: The group of adjacent organs consisting of prostate, urinary bladder, seminal vesicles and coagulation glands is removed (see figures 4.4.d through 4.4.f) and (if weights are not required) fixed in toto to prevent leakage of the glandular secretions.

After fixation, the ventral lobe is detached from the urinary bladder and is flipped back. The urinary bladder and seminal vesicles with coagulation glands are removed. The two ventral lobes are separated from each other, but are left attached to the dorsolateral parts. The dissected prostate is put into a cassette with the “outer” surfaces down; i.e. ventral face of the ventral lobes down and dorsal face of the dorsolateral lobes down (see figures 4.4.g through 4.4.i). After histotechnical processing, a section at the mid level of the ventral lobes is made.

The dorsocranial lobe of the prostate (i.e. coagulating gland) is processed with the seminal vesicle.

Chemically induced or spontaneous proliferative lesions of the rat prostate can be found in all three lobes. The dorsal and lateral lobes exhibit the same spectrum of proliferative lesions. These differ from spontaneous and induced lesions in the ventral lobe. Additionally, some strain specific deviations in the interlobular distribution of benign and malignant neoplasms consequently require the assessment of all compartments. Accordingly, a longitudinal-horizontal section through the prostate complex, including dorsolateral and ventral lobes, urethra and, optionally, ureter and ductus deferens represents a less time consuming method, applicable to routine histological processing and examination.

Related references

Fig. 4.4c. Prostate.

Abbreviations used in figures 4.4c to 4.4h:
Cg: Coagulation gland
Dd: deferent duct
Dl: dorsolateral lobe of prostate
Dsv: Duct of seminal vesicle
Sv: Seminal vesicle
Ub: Urinary bladder
Ur: Urethra
Vl: ventral lobe of prostate
**Fig. 4.4d.** Rat, abdominal cavity, ventral aspect. In-situ localization of prostate and attached organs.

**Fig. 4.4e.** The urethra is dissected.

**Fig. 4.4f.** Removal of prostate, urinary bladder, and seminal vesicles as a unit.

**Fig. 4.4g.** “Outer” aspects of freshly dissected (left) and fixed (right) prostate.

**Fig. 4.4h.** “Inner” aspects of fixed (left) and freshly dissected (right) prostate. Compared to the situation in the living animal (in situ), the ventral prostate is flipped back.

**Fig. 4.4i.** The prostate lobes are embedded with the “outer” aspects down, i.e. the dorsolateral lobes with the dorsal surface down, and the ventral prostate with the ventral surface down, because this part was flipped back.
5 Female genital system
5.1 Ovary and Oviduct

Number of sections: 2 (1 per side and organ)
Direction: Ovary: longitudinal
Oviduct: transverse
Remarks:

If ovaries are not weighed:
Ovary processed along with the oviduct. Longitudinal sections are made, resulting in multiple transverse sections of the oviducts.

If ovaries are weighed:
Ovaries and oviducts are separated at necropsy.

In rats, the ovary is embedded together with the oviduct and a central section is cut. Larger ovaries or ovaries with masses are halved longitudinally or a slice from the middle of the organ is taken at trimming. In mice, the ovary is very small and therefore removed and fixed together with the bursa to avoid preparation artifacts. This procedure allows the detection of bursa cysts and cystadenomas in mice. Ovarian cysts should remain intact if possible.

If the oviduct is prepared attached to the ovary, the longitudinal cut through the ovary will result in multiple transverse sections through the oviduct.

If the ovaries are weighed, attached tissues (ovarian bursa, oviduct) have to be removed. In this case, the oviduct together with the tip of the uterine horn is dissected from the ovary during necropsy. The whole oviduct in conjunction with the tip of the uterine horn is fixed and embedded. Longitudinal sections through the tip of the uterine horns are made, resulting in transverse sections of the oviduct.

If the uterus is also weighed, the oviducts are detached from the horns and put into a cassette with the ovaries.

Related references

Fig. 5.1a. Ovary and oviduct, if ovaries are not weighed.

Fig. 5.1b. Ovary (Ov), oviduct (Od) and uterine horn (H), if ovaries are weighed.

Fig. 5.1c. Ovaries (top) and oviducts (bottom). Ovarian bursa removed, oviducts separated from ovaries and uterine horns.
5 Female genital system

5.2 Uterus and Vagina

Localizations:
1 + 2) Middle region: uterine horns
3) Whole organ: uterine body and cervix
4) Whole organ or anterior portion: vagina

Number of sections:
3, 4 if uterus is separated from vagina

Direction:
1 + 2) Transverse: uterine horns
3) Longitudinal horizontal: uterine body (corpus), cervix and vagina

Remarks:
Optional: oviducts together with the tip of the uterine horns; other options see ovary.

The uterine body (fused part of the uterus) together with the vagina should be placed with its dorsal aspect on cardboard before fixation. If uterus is weighed, uterine cervix and vagina are two separate specimens which are cut longitudinally.

A horizontal section is made through the cervix and vagina. A transverse section is made through the middle portion of both uterine horns. These sections cover the relevant anatomical and functional structures of these organs. In most cases, the uterine body and most of the vagina will fit in one cassette, facilitating the interpretation of findings in the female genital tract.

The oviducts are fixed attached to the uterus when the ovaries are weighed.

Related references

Fig. 5.2a. Uterus and vagina, ventral aspect.

Fig. 5.2b. Uterus and vagina (V: vagina, C: cervix, B: body, H: uterine horn, Od: oviduct, Ov: ovary).

Fig. 5.2c. Uterine horn.

Fig. 5.2d. Uterine cervix.

Fig. 5.2e. Vagina.
6 Endocrine system
6.1 Pituitary gland

Localization: Sella turcica of sphenoid bone
Number of sections: 1
Direction: Transverse, parallel to the caudodorsal surface of the gland.
Remarks: To avoid destruction of the pituitary, fixation is recommended before removal from the skull and/or weighing.

The organ is embedded in toto with its caudodorsal surface down, so that the section will include all three parts.

Optional: trimmed in situ, closely caudal to the pituitary, caudal surface embedded down.

Transverse section of decalcified skull.

The pituitary consists of three portions: pars distalis, pars intermedia, and pars nervosa. All three parts should be present in one histologic section with the largest possible area.

Relevant differences between rats and mice

In mice, in situ fixation may be particularly useful to avoid preparation artifacts and to obtain consistent planes of section (Mahler and Elwell, 1999).

Related references

Fig. 6.1a. Pituitary gland, in situ localization, median aspect (Pn: pars nervosa, Pd: pars distalis, Sb: sphenoid bone).

Fig. 6.1b. Skull, dorsal view. Level for optional in-situ trimming.

Fig. 6.1c. Pituitary gland in situ (Pn: pars nervosa, Pi: pars intermedia, Pd: pars distalis, Sb: sphenoid bone).

Fig. 6.1d. Pituitary gland, male rat, immunohistochemical staining for prolactin: depending on the level of section, the positive cells are not evenly distributed.
6 Endocrine system
6.2 Thyroid gland, Parathyroid gland, Trachea (oral study) and Esophagus

Localization: In the area of the parathyroid gland
Number of sections: 1
Direction:

If thyroid glands are not weighed:
Transverse section of trachea, esophagus, thyroid and parathyroid glands.
Optional: longitudinal horizontal section of thyroid glands in conjunction with trachea. A separate transverse section of the esophagus is made.

If thyroid glands are weighed:
Longitudinal horizontal (largest cut surface), section of thyroid and parathyroid glands.
Transverse section of trachea and esophagus.

Remarks: Recuts are sometimes required to consistently include the parathyroid in the section. The number of focal lesions observed depends on the area of thyroid examined.

Relevant differences between rats and mice
The rat possesses only one pair of parathyroids. They are located on the anterior and lateral aspect of the thyroid lobes but may vary in position.

In the mouse, the position and the number of parathyroids is variable. Usually, there are two parathyroid glands located bilaterally just under the capsule near the dorsolateral border of each thyroid lobe. They are rarely found at the same level, sometimes one or both may be posterior to the thyroid; they may be deeply embedded in the thyroid tissue and there may be more than two.

Related references

Fig. 6.2a. Transverse section.
Fig. 6.2b. Transverse section (T: trachea, Tg: thyroid gland, Pg: parathyroid gland, E: esophagus).
Fig. 6.2c. Thyroid gland, longitudinal section, with parathyroid gland (Pg).
6 Endocrine system
6.3 Adrenal gland

Localization: Through cortex and medulla
Number of sections: 2 (1 per side)
Direction: Longitudinal (largest cut surface)
Remarks: Embedded in toto; careful microtome sectioning until recommended cutting level is obtained.

Median sections of the adrenal glands (one per side) are required in order to demonstrate a representative part of both cortical and medullary tissues.

Relevant differences between rats and mice
The mouse adrenal differs from that of the rat by the absence of a zona reticularis in the inner cortex and an additional “X-zone” at the junction between cortex and medulla in females which regresses with age (Nyska and Maronpot, 1999). The adrenal gland in male mice is very small. Therefore, careful cutting is required to obtain medullary tissue in the section.

Related references

Fig. 6.3a. Adrenal gland.

Fig. 6.3b. Adrenal glands.

Fig. 6.3c. Adrenal gland, male mouse (C: cortex, M: medulla).

Fig. 6.3d. Adrenal gland, female mouse, age about 3 months (C: cortex, M: medulla, X: X-zone).
References


HARKEMA JR: Comparative pathology of the nasal mucosa in laboratory animals exposed to inhaled irritants. Environ Health Perspect 1990; 85: 231–238.


Revised guides for organ sampling and trimming in rats and mice – Part 3

A joint publication of the RITA*) and NACAD**) groups

GERD MORAWIETZ1, CHRISTINE RUEHL-FEHLERT2, BIRGIT KITTEL3, AXEL BUBE4, KEVIN KEANE5, SABINE HALM6, ANKE HEUSER7, and JÜRGEN HELLMANN8

With 48 figures

Received: March 25, 2004; Revised; May 17, 2004; Accepted: May 25, 2004

Address for correspondence: GERD MORAWIETZ, Department of Information Technology and Databases, Fraunhofer Institute of Toxicology and Experimental Medicine, Nikolai-Fuchs-Str. 1, 30625 Hannover, Germany; Fax: ++49 511 5350 155, E-mail: morawietz@item.fraunhofer.de

Key words: Trimming; RITA; NACAD; rat; mouse; standardization; guidelines; kidney; ureter; urinary bladder; brain; spinal cord; spinal nerve root; eye; optic nerve; Harderian gland; skeletal muscle; peripheral nerve; bone; cartilage; femur; joint; heart; aorta; thymus; spleen; bone marrow; sternum; lymph nodes.

Summary

This is the third part of a series of three articles on trimming instructions of rat and mouse protocol organs and tissues in regulatory type toxicity studies, covering the urinary, nervous, musculoskeletal, cardiovascular, and lymphoreticular systems. The article is based on the experience of the European RITA and American NACAD working groups and is an extended revision of trimming guides published in 1995 (BAHNMANN et al.). The optimum localization for tissue preparation, the sample size, the direction of sectioning and the number of sections to be prepared is described organ by organ. These descriptions are illustrated for each organ by a schematic drawing and/or a macro-photograph showing the plane of section as well as a low magnification of the H&E stained slide demonstrating the optimum “end-product”.

*) RITA: Registry of Industrial Toxicology Animal-data. Members: Abbott GmbH & Co KG, Ludwigshafen, Germany; ALTANA Pharma AG, Hamburg, Germany; AstraZeneca, Södertälje, Sweden and Macclesfield, England; Aventis Pharma Deutschland GmbH, Hattersheim, Germany; BASF AG, Ludwigshafen, Germany; Bayer HealthCare AG, Wuppertal, Germany; Boehringer Ingelheim Pharma GmbH & Co KG, Biberach, Germany; Fraunhofer Institute of Toxicology and Experimental Medicine, Hannover, Germany; Hoffmann-LaRoche AG, Basel, Switzerland; Merck KGaA, Darmstadt, Germany; Novartis Pharma AG, Basel, Switzerland; Pfizer, Amboise, France; Pharmacia, Nerviano, Italy; Syngenta CTL, Macclesfield, England

**) NACAD: North American Control Animal Database. Members: 3M Corporate Toxicology, St. Paul, MN, USA; Adolor Corporation, Malvern, PA, USA; Bayer CropScience, Stillwell, KS, USA; Pfizer, Inc., Groton, CT, USA; Pfizer, Inc., Ann Arbor, MI, USA; Pfizer, Inc., Kalamazoo, MI, USA; Schering-Plough Research Institute, Lafayette, NJ, USA
The objectives of this work, as addressed in detail in the first part (RUEHL-FEHLERT et al. 2003), are to standardize tissue sampling and trimming for comparison of historical data obtained from different studies and different laboratories, ensure the presence of all relevant target sites for histopathological evaluation and provide technical advice for preparatory techniques during necropsy, fixation and trimming (CRISSMAN et al. 2004).

**Brief introduction to the use of the individual organ guides**

For each organ the following information is usually given (for more details see part 1: RUEHL-FEHLERT et al. 2003):

1. **Localization:** anatomical site or part of an organ from which a sample should be taken (i.e. lobe).
2. **Number of samples:** number of organs (i.e. both for bilateral organs) or organ pieces prepared for evaluation (not necessarily identical with the number of slides/blocks).
3. **Direction:** direction (plane of section) in which an organ should be cut at trimming or microtome sectioning. The proposed plane of section is shown in green color and optional sections (if defined) are shown in blue (see fig. 1 for an explanation of the symbols used).
4. **Sample size:** the size (area) of an organ or part of an organ which is placed in a cassette for processing. The sample size is determined by the size of the organ. For optimal fixation, sample thickness should not exceed 3–5 mm. In general, the examined area should be as large as possible and should contain the relevant anatomical structures. The tissue can be adapted to the size of cassettes by trimming the margins off.
5. **Optional remarks** are used to present additional information, as recommended by the RITA/NACAD groups, such as the instillation of fixative into the lung or the urinary bladder, reasons for optional recommended sections, placing of organs in cassettes, etc.
6. **Schematic drawings** and/or **gross photographs** are given indicating the plane of section. Some of the gross photographs show the organ and trimming direction *in situ* for orientation purposes. However, it is recommended to remove the organ or tissue first.Trimming can then be performed on the fresh wet tissue or, in most cases, after fixation of the organ. Most of the gross photographs were taken from fresh unfixed organs; shape and color may be slightly different after fixation.

7. An image of a Hematoxylin and Eosin (H&E) stained slide is shown for the recommended section level (sometimes also for optional levels). Typical structures included in this section are indicated as necessary. If not otherwise specified, 10% buffered formalin is recommended as the fixative.

The following terms are used to describe the trimming directions (see also fig. 2 with a schematic presentation of the related cut levels):

- **transverse:** perpendicular to the long axis of an organ or part of an organ
- **longitudinal vertical:** in the direction of the long axis of the body, an organ or part of an organ in the dorsoventral axis or parallel to it (in the text also referred to in short as “longitudinal”)
- **longitudinal horizontal:** in the direction of the long axis of the body, an organ or part of an organ, perpendicular to the dorsoventral axis (in the text also referred to in short as “horizontal”)

By defining either the “body”, the “whole organ” or a “part of an organ” (for example a liver lobe or a certain part of the brain), as a unit of reference, it is relatively simple to precisely characterize a trimming direction by using only the three above defined terms and avoiding therefore the vast amount of anatomical terms and confusing synonyms present in literature.

**Final technical remarks**

**Study types:** Although carcinogenicity studies in rodents are the focus of this publication, the same trimming procedures are also recommended for short-term rodent studies, unless specific target tissues or organs require an adaptation. In general, it is advisable to follow one standard trimming procedure in the laboratory for all studies to avoid technical inconsistencies and to facilitate inter-study comparison.

**Fig. 1.** Symbols used in the drawings and/or gross photographs to indicate the plane of section. *a:* cutting level parallel to the plane of the picture, *b:* cutting level perpendicular to the plane of the picture, *c:* cut level, 3-D.

**Fig. 2.** Schematic presentation of the plane of section. *a:* transverse, *b:* longitudinal vertical, *c:* longitudinal horizontal.
**Additional organs:** Besides the organs addressed in these guides, special investigations or guidelines may require additional organs. Some can be found in the specimens described here, such as teeth in the transverse sections of the nasal cavity, brown adipose tissue at the renal hilus and heart base, white adipose tissue in the subcutis and the urethra of males in the prostate section. Others, however, may need adaptation of sampling procedures or collection of additional specimens.

**Blocking:** The following recommendations should be taken into account:

1. For reasons of economy it is desirable to have a small number of blocks per animal.
2. Unduly large numbers of specimens in one block can lead to a loss of quality. Besides the obvious limitation of specimen size, difficulties may arise when cutting tissues with different physical properties in the same block.
3. Small organs often benefit from being embedded by themselves which makes it easier to cut accurately the level of interest. Examples are the pituitary gland, the adrenal gland and the thyroid gland with parathyroid glands.
4. Organs with similar cutting properties are often combined in one block.
5. The pathologist benefits from being able to examine organs in functional groups, e.g. stomach together with intestine or a combination of the lymphoid organs in one block.

In order to reduce the number of slides, more than one block may be placed on a slide. Some laboratories see an advantage in collecting the adrenal glands, pituitary gland and thyroid gland with parathyroid glands on one slide which were processed in different blocks. An example of a blocking scheme reflecting the above mentioned considerations was given by Krinke (2000).

**Number of sections:** The number of sections per specimen is usually one. If in some instances more than one section has to be taken, it should be borne in mind to evaluate the same amount of sections in all animals/groups to obtain comparable results.

**Staining:** The H&E stain is regarded as the standard in toxicological studies. Other histological stains and immunohistochemistry can be applied as a routine or on a case by case basis in addition to the H&E stained sections.

**Fixation:** In literature a volume ratio of tissue to fixative of 1:20 is often mentioned. However, much less fixative is sufficient, especially if a shaking device is used for freshly fixed tissues and/or fixative is replaced once. Tissues must be promptly and appropriately fixed by immersion. Adequate fixation time is necessary before tissue processing commences (Crissman et al. 2004).

**Internet:** With kind permission of Elsevier Publishing Jena, an extended version of these guides for organ sampling and trimming will be available in the Internet under the URL www.item.fraunhofer.de/reni/trimming.

**Acknowledgements:** The authors would like to thank Mr. Theodor Leforeme and Mrs. Brigitte Poschmann (Bayer HealthCare AG) for the gross preparation of organs and histological slides; Mrs. Petra Hartmann, Mrs. Mieke Petersen and Mrs. Martina Neuenhaus (Bayer HealthCare AG) for scanning the histological slides, Dr. Gerd-Peter Fehlert for his help in image processing; Dr. Wolfgang Kaufmann (BASF AG) for his scientific advice regarding the nervous system, Dr. Paul Deslex (Pfizer Centre Recherche) for providing additional photographs and slides, Dr. Thomas Nolte (Boehringer Ingelheim Pharma GmbH & Co KG) and Dr. Anne Provencher Bolliger (Novartis Pharma AG) for help in establishing the bone marrow manuscript and Dr. Matthew Jacobson (Syngenta CTL) and Dr. Richard Doughty (AstraZeneca) for their helpful input during the editing process.

**References**


7 Urinary system
7.1 Kidney and Ureter

Localizations:
- Kidney: both in the median, through the tip of papilla and renal pelvis
- Ureter: transverse section midway between kidneys and bladder.
- Optional: adjacent to the renal pelvis (not shown in the image)

Number of sections: 2 (1 per side)

Direction:
- Kidney: one side longitudinal, other side transverse
- Ureter: longitudinal adjacent to kidney or transverse with adipose tissue

Remarks kidney:
- Capsule should not be removed.
- Fixation can be improved by an incision at necropsy.

The transverse section of the kidney from the middle portion allows optimal examination of the renal pelvis, renal papilla and the junction with the ureter. The longitudinal section permits histological evaluation of a relatively large area of tissue which includes both renal poles. This is advantageous for the evaluation of any focal lesions. In addition, the regions of the renal pelvis close to the poles are of interest with respect to concretions and urothelial changes.

A slightly paramedian cut at trimming is helpful to get the full length of the renal papilla in section.

The ureter can be fixed on a cardboard together with a small amount of attached adipose tissue.

Related references

Fig. 7.1a. Kidney and ureter.

Fig. 7.1b. Kidney and ureter (U) in situ.

Fig. 7.1c. Kidneys (P: papilla, M: medulla, C: cortex, Rp: renal pelvis).

Fig. 7.1d. Ureters.
7 Urinary system

7.2 Urinary bladder

Number of sections: 1
Direction: Longitudinal vertical
Remarks: Instillation 0.2 ml rat, 0.05 ml mouse

For instillation, the needle is inserted via the urethra into the urinary bladder. For the mouse, fixative can be injected through the urinary bladder wall after ligation of the urethra. The organ is filled by injection of approximately 0.2 ml (rat) or 0.05 ml (mouse) of the fixative. Do not infuse if distended with urine. Ligation is performed with a ventral knot. In addition, it may be helpful to mark the ventral side of the bladder with a stick of silver nitrate. After removal of the bladder, fixation is continued in a suitable container. The bladder is cut vertically through the ventral knot to access the following regions:

1. Vertex and ventral body: The vertex is the area generally most prone to develop neoplasms; deposition of sediments and calculi occurs mainly in the ventral aspect, which can lead to urothelial alterations.
2. Dorsal part of the bladder.
3. Bladder neck with trigone: Whilst most of the urinary bladder in the embryo is of endodermal origin, the trigone is derived from the mesoderm of the Wolffian ducts.

Related references

Fig. 7.2a. In situ localization and fixation of urinary bladder.

Fig. 7.2b. Instillation procedure: in situ localization, ventral aspect, after opening of the abdominal cavity. P: prostate, R: rectum, S: seminal vesicle, U: urinary bladder.

Fig. 7.2c. Urinary bladder after instillation.
8 Nervous system

8.1 Brain

Localizations:  
1) Cerebrum at the optic chiasm  
2) Cerebrum at the base of the posterior hypothalamus  
3) Midcerebellum and medulla oblongata  
Optional:  
4) Pons at the middle of its protrusion  
5) Cranial cervical cord  

Number of sections: 3 (4)

Direction: Transverse

Remarks: Embedded with the anterior faces down.  
To achieve accurate brain weights, the spinal cord should be cut off at a consistent level.

Unless the scope of examinations in neurotoxicity studies is extended, it is advised to use the above mentioned brain sections for the morphological screening in all rodent studies concerned.

Transverse sections of the brain are required to assess whether the findings are distributed uni- or bilaterally and symmetrically or asymmetrically. The areas of the brain known to be susceptible to neurotoxicity, including oncogenicity, are: the cerebral and cerebellar cortex, hippocampus, basal ganglia, thalamus, hypothalamus, midbrain, pons and medulla oblongata. Routinely, three transverse sections are obtained:

1. the first section at the level of optic chiasm including the basal ganglia, septum, cortex, anterior hypothalamus;
2. the second section at the level of hippocampus containing the cortex and brain stem at the transition of diencephalon to mesencephalon and
3. the third section containing the cerebellum and brain stem (medulla oblongata).

Location 4 is recommended for special investigations of the pons.

To achieve precise vertical cuts, brain matrix moulds can be used. Different sizes are available for different laboratory species. For rodents, the brain can be sliced at 1 mm intervals.

A sample of the cranial cervical cord can be removed together with the brain (location 5). Alternatively, it can be taken together with the cranial vertebral column to avoid artifacts (see 8.2: spinal cord).

Trimmed specimens should not be stored in alcohol for extended periods during routine processing to avoid artifactual vacuolation.

Related references
Abbreviations used in figures 8.1d to 8.1g:
Ac: anterior commissure
C: cerebellum
Ce: cerebral cortex
Cea: corpus callosum
H: hypothalamus
Hc: hippocampus
Mo: medulla oblongata
O: optic chiasm
P: pons
Sr: striatum
T: thalamus

Fig. 8.1d. Brain, localization 1, cerebrum.

Fig. 8.1e. Brain, localization 2, cerebrum.

Fig. 8.1f. Brain, localization 3, cerebellum and medulla oblongata.

Fig. 8.1g. Brain, optional localization 4, cerebrum and pons.
8 Nervous system

8.2 Spinal Cord and Spinal nerve root

Localizations:
1) Cervical cord at upper cervical segment. *Optional:* cervical cord cut at the level of the first cervical nerve (see 8.1 brain)
2) Thoracic cord at mid-thoracic segment
3) Lumbar cord at 4th lumbar segment close to last rib

Number of sections: 3
Direction: Transverse
Remarks: In conjunction with vertebral body (decalcified). *Optional:* without bone to avoid decalcification. Embedded with anterior faces down.

Transverse sections of the spinal cord are required to assess whether the findings are distributed uni- or bilaterally, and symmetrically or asymmetrically. Sections should be obtained at the upper cervical, mid-thoracic and lumbar levels. The upper cervical area positioned at the transition of medulla oblongata to cervical spinal cord (level of the first cervical nerve) is important for the detection of distally accentuated neuropathy affecting the long spinal tracts. This region is known to produce the earliest and most prominent lesions in the dorsal cervical column. At necropsy, the spinal cord should be cut off directly posterior to the cerebellum and can be removed with the vertebral bone as with the thoracic and lumbar spinal cord.

In the lumbar spinal cord, the area of L4 segment should preferably be examined, as this segment provides the main contribution to the peripheral sciatic nerve. The L4 segment of the spinal cord is located at the junction of the thoracic and lumbar spine which is indicated by the last rib. If the lumbar specimen is taken more caudally, it will contain only the cauda equina.

It is advantageous to remove the spinal cord from the vertebrae before processing to avoid decalcification. However, processing *in situ* has the advantage to include dorsal root ganglia and nerve roots to assess radiculoneuropathy.

In neurotoxicity studies, sections from the cervical and lumbar swellings are recommended in current guidelines (OECD 424). The cervical swelling is located at the C3–C6 segments of the spinal cord. This section is not prioritized but can be taken as additional option in routine rodent studies.

To avoid artifactual vacuolation in the white matter, the specimens should not be stored in alcohol.

---

**Related references**

![Fig. 8.2a. Spinal cord.](image1)

![Fig. 8.2b. Spinal cord (Cc: cervical cord, Tc: thoracic cord, Le: lumbar cord). Option without bone shown for the cervical cord.](image2)
8 Nervous system
8.3 Eye, Optic nerve and Harderian gland

Localization: In the plane of the optic nerve
Number of sections: 2 (1 per side)
Direction: Longitudinal vertical
Remarks: Optic nerve included.
Optional: together with Harderian gland.
Optional: together with eye lids for better orientation.
For long-term studies, formalin fixation is generally sufficient. For other study types, fixation in Davidson’s fixative is recommended to avoid detachment of the retina.

Each eye can be removed from the socket together with the optic nerve and the Harderian gland (optional) after fixation of the head. These organs are embedded in such a way that the section plane includes the anterior pole of the eye, the lens, the optic nerve and the gland.
Alternatively, each eye with the optic nerve can be taken at necropsy followed by the removal of the Harderian gland; in this case, both samples are fixed and embedded separately.
As an option, the optic nerve specimen can be taken at the base of the brain.

Relevant differences between rats and mice
The rodent eye has no areas of increased visual acuity (fovea and macula), therefore orientation is not as critical as in the non-rodent eye. The Harderian gland lies intraorbitally and is cone-shaped in the rat and horseshoe-shaped in the mouse.

See also: 2.2 Salivary glands (extraorbital lacrimal gland)

Related references

Fig. 8.3a. Eye, optic nerve and Harderian gland.
Fig. 8.3b. Eye.
Fig. 8.3c. Eye and eye lid.
Fig. 8.3d. Eye, Davidson’s fixative (O: optic nerve, H: Harderian gland).
Fig. 8.3e. Harderian gland.
Fig. 8.3f. Optic nerve, taken from the brain near to the optic chiasm.
8 Nervous system
8.4 Skeletal muscle and peripheral nerve

Localizations: Biceps femoris muscle
Sciatic nerve
Number of sections: 3 (2)
Direction: Skeletal muscle: longitudinal and/or transverse
Sciatic nerve: longitudinal; optional: transverse
Remarks: Muscle and nerve are sampled separately.
Optional: skeletal muscle and sciatic nerve sampled attached to each other.
Longitudinal and transverse sections in one cassette.
If only one sample of muscle is processed, a transverse section is preferred.

If skeletal muscle and sciatic nerve are sampled together, the gracilis, adductor, semimembranous and semitendinous muscles are removed from the medial aspect of the thigh to get access to the sciatic nerve running along the medial surface of the biceps femoris muscle. The sample is taken by proximal and distal transverse cuts. After fixation, transverse and longitudinal sections are prepared.

The sciatic nerve can be fixed on a cardboard, if not sampled together with muscle.

Related references
McGavin 1991, Popesko et al. 1992

Fig. 8.4a. Preparation of skeletal muscle and sciatic nerve from the hind leg.

Fig. 8.4b. Skeletal muscle and sciatic nerve, transverse and longitudinal horizontal sections.

Fig. 8.4c. Muscle in situ, option.

Fig. 8.4d. Skeletal muscle, longitudinal section.

Fig. 8.4e. Skeletal muscle, transverse section.

Fig. 8.4f. Sciatic nerve, longitudinal section.
9 Musculoskeletal system
9.1 Bone, Cartilage, Femur and Joint

Localization: Knee joint with distal femur and proximal tibia
Number of sections: 1
Direction: Longitudinal
Remarks: Decalcified

For routine histological examination of bone (and bone marrow), the distal portion of one femur with the knee joint and proximal portion of the adjacent tibia are removed at necropsy, fixed and decalcified. A longitudinal section is then made through femur, knee joint and tibia, possibly including patella and/or menisci. Both long bones should be cut at similar length with inclusion of parts of their diaphyses. The section should be slightly lateral to the center of the joint to ensure that articular cartilage is present rather than ligaments. The use of large sections of bone with the joint has the advantage of maintaining anatomic integrity and allows separation of systemic pathologic conditions from reactive processes. With the proposed technique, the epiphyses, metaphyses, growth plates and articular cartilages of femur and tibia are also sectioned allowing assessment of growth, modeling, and remodeling parameters.

In old rats, the femur contains a high proportion of fat marrow.

Related references
WOODARD et al. 2002

Fig. 9.1a. Femur and tibia.

Fig. 9.1b. Femur (F) and tibia (T). Picture taken from a young rat.
10 Cardiovascular system

10.1 Heart

Localization: Through ventricles and atria with auricles
Number of sections: 1
Direction: Longitudinal
Remarks: One half that contains the main vascular trunks

A longitudinal section through both ventricles should be made from the base to the apex of the heart. Do not open the heart at necropsy. The half with the main vessel trunks is blocked to get a section through the opened ventricles and atria with auricles as well as through base, septum, apex, papillary muscle and main vessels of the heart.

Related references

![Fig. 10.1a. Heart (Ao: aorta, Cv: conoventricular vein, La: left auricle, Lv: left ventricle, Ra: right auricle, Rv: right ventricle).](image)

![Fig. 10.1b. Heart (Ao: aorta, At: atrium, Lv: left ventricle, Rv: right ventricle).](image)

![Fig. 10.2a. Aorta, ventral view.](image)

![Fig. 10.2b. Aorta, dorsal view.](image)

![Fig. 10.2c. Aorta and adjacent brown adipose tissue (At).](image)

10 Cardiovascular system

10.2 Aorta

Localization: Thoracic region
Number of sections: 1
Direction: Transverse

The section should be taken from the middle of the last 1 cm caudal segment of the thoracic aorta. This region is closely attached to the thoracic vertebrae and can easily be removed during necropsy.

Induced lesions of the vascular system are rare in rats except for some pharmaceutical compounds such as vasodilators. Most of the induced changes are observed in smaller arteries and arterioles of various organs but not in thick-walled major blood vessels such as the aorta.

Related references
VAN VLEET et al. 2002
11 Lymphoreticular system

11.1 Thymus

Localization: Along the length of one lobe
Option: whole organ
Number of sections: 1
Direction: Longitudinal
Remarks: Largest area
Thymic region in old animals

The whole thymus is fixed and trimmed along the length of one lobe. This gives a standardized longitudinal section showing all anatomical structures of this organ.

In immuno-toxicological assays, it is advisable to embed the whole organ, ventral aspect down, and to cut both lobes. In case of thymic atrophy or involution, the whole organ/thymic region should be embedded.

Fig. 11.1a. Thymus, young rat.

Fig. 11.1b. Thymus (C: cortex, M: medulla).

Fig. 11.1c. Atrophic thymus.

Related references
11 Lymphoreticular system

11.2 Spleen

Localization: At largest extension
Option: whole organ
Number of sections: 1
Direction: Transverse
Option: longitudinal horizontal (not shown in the image)

A transverse section is made at the largest extension of the organ, showing red and white pulp. This plane of section guarantees the presence of all relevant anatomical structures and hallmarks of the white pulp, e.g. PALS (periarteriolar lymphatic sheath), marginal zone and follicles.

Related references

Fig. 11.2a. Spleen.

Fig. 11.2b. Spleen (H: hilus).

11.3 Bone marrow (Sternum)

Localization: Sternum
Number of sections: 1
Direction: Longitudinal horizontal
Sample size: 2–3 sternebrae
Remarks: Decalcified

The bone marrow is generally examined concurrently with the bone tissue. From this decalcified section it is possible to evaluate the cellularity, number of megakaryocytes and the stromal compartment. Examination of a bone marrow smear of a core sample from the femur may be useful for evaluation of iron content and more precise cytology.

Related references

Fig. 11.3a. Sternum with bone marrow.

Fig. 11.3b. Sternum with bone marrow.
11 Lymphoreticular system
11.4 Bone marrow smear

Localization: Core sample from the femoral diaphysis
Number of smears: 1
Remarks: Slides should only be stored after fixation in methanol. Staining with the Pappenheim, May-Gruenwald-Giemsa or Wright method.

Various techniques have been described. For each method training is necessary to obtain satisfactory smears. Femur diaphysis is recommended as localization because no bone trabecula are intermingled with the hematopoietic tissue.

The proximal and distal epiphyses are cut off with scissors. For removal of the bone marrow, air is blown from one end into the marrow cavity and the marrow cast is collected onto a glass slide. The smear is prepared conventionally with a cover glass. A smear of adequate quality contains grossly visible particles. Marrow smears should be prepared as fresh as possible to avoid blood clotting.

For collection of the bone marrow, aspiration with a pipette containing anticoagulated serum or a small paint brush or a cotton bud from the longitudinally opened femur can also be used.

Related references
VALLI et al. 1990, VALLI et al. 2002

Fig. 11.4a. Low magnification of a rat bone marrow smear, stained with May-Gruenwald-Giemsa, showing grossly visible particles (G) and regions suitable for evaluation (R).

Fig. 11.4b. High power view of a rat bone marrow smear stained with May-Gruenwald-Giemsa.
11 Lymphoreticular system

11.5 Lymph nodes

Localization: Mesenteric lymph node
Optional: mandibular lymph node, axillary lymph node, popliteal lymph node, auricular lymph node, inguino-femoral lymph node
Inhalation study: lung associated lymph nodes

Number of sections: 1 (per lymph node)
Direction: Longitudinal (largest cut surface)
Sample size: Whole organ
Remarks: In case of parenteral application, one lymph node draining the application site and another distant from it should be chosen

The peripheral lymph nodes that are most often examined are the mandibular, axillary and/or popliteal lymph nodes. The lymph nodes are often embedded untrimmed as whole organ due to their small size. It is important that a section is taken through the middle of the longitudinal axis of the lymph node in order to be able to examine all major areas, such as cortex, paracortex and medulla.

Related references

References for organ guides


