Revised guides for organ sampling and trimming in rats and mice – Part 1
A joint publication of the RITA*) and NACAD**) groups

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With 51 colored figures

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*) RITA: Registry of Industrial Toxicology Animal-data. Members: Abbott GmbH & Co KG, Ludwigshafen, Germany; ALTANA Pharma AG, Ludwigsafen, Germany; Astrazeneca, Södertälje, Sweden and Macclesfield, England; Aventis Pharma Deutschland GmbH, Hattersheim, Germany; BASF AG, Ludwigshafen, Germany; Bayer AG, Wuppertal, Germany; Boehringer Ingelheim Pharma GmbH & Co KG, Biberach, Germany; Fraunhofer Institute of Toxicology and Experimental Medicine, Hannover, Germany; Hoffman-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; Pharmacia, Inc., Kalamazoo, MI, USA; R.W. Johnson Pharmaceutical Research Institute, Spring House, PA, USA; Schering-Plough Research Institute, Lafayette, NJ, USA

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 (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 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; BREIGMAN 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 instillation 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. 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

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**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).

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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

Fig. 1.1a. Skin, transverse and longitudinal cutting direction.
Fig. 1.1b. Skin, inguinal region: transverse and longitudinal cutting direction.
Fig. 1.1c. Skin and mammary gland (M), section transverse to the direction of the hair flow.
Fig. 1.1d. Skin, longitudinal section in the direction of the hair flow.
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 (anterior-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

Fig. 2.3a. Larynx (dorsal view) indicating the level in the region of the ventral pouch.

Fig. 2.3b. Larynx (L) (with ventral pouch and vocal processes) and pharynx (P).

Fig. 2.4a. Tongue base and cranial esophagus/trachea: esophagus (E) and trachea (T) with tongue (To), hyoid bone (H) and thyroid gland (Th).

Fig. 2.4b. Esophagus (E) and trachea (T) at the level of the thyroid glands.
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.

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

Fig. 2.7b. Rat liver, visceral aspect.

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

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


