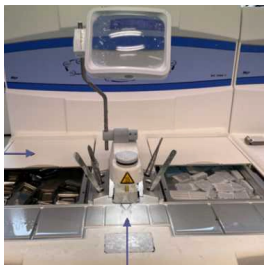
May 07, 2024

Histology examination: preparation and staining of bovine tissues



DOI
dx.doi.org/10.17504/protocols.io.4r3l22wn3l1y/v1

Jade Ros[1], Marthe Vilotte[1], Julie Rivière[1,2], Cecile Grohs[1]

[1]Université Paris-Saclay, INRAE, AgroParisTech, GABI, 78350, Jouy-en-Josas, France;
[2]Université Paris-Saclay, INRAE, AgroParisTech, MICALIS, 78350, Jouy-en-Josas, France

Cecile Grohs
Université Paris-Saclay, INRAE, AgroParisTech, GABI, 78350, ...

DOI: dx.doi.org/10.17504/protocols.io.4r3l22wn3l1y/v1

Protocol Citation: Jade Ros, Marthe Vilotte, Julie Rivière, Cecile Grohs 2024. Histology examination: preparation and staining of bovine tissues. **protocols.io https://dx.doi.org/10.17504/protocols.io.4r3l22wn3l1y/v1**

Protocol status: Working
We use this protocol and it's working

Created: February 15, 2024

Last Modified: May 07, 2024

Protocol Integer ID: 95291

Keywords: Histology, HE staining, embedding tissue, scanning histological slides

Abstract

Histology is a technique that makes it possible to highlight what we want to observe and to "fix" a sample at a given time, while preserving characteristics close to its living state (fixation). For example, some elements, cells or cellular organelles can be stained differently depending on the research objective, which may require the detection of certain chemical compounds (histochemistry).
Hematoxylin and Eosin staining is the most common staining to observe the morphology of a tissue. Hematoxylin is used to illustrate nuclear detail in cells. Eosin allows identifying the cytoplasmic component of the section.

All manipulations described in this protocol were performed on bovine samples at the @BRIDGe (Animal Biological Resources for Integrated and Digital Genomics) platform of the UMR GABI, Université Paris Saclay, INRAE, AgroParisTech, GABI, 78350, Jouy-en-Josas, France.

Image Attribution

All photos used in this protocol were taken by Jade Ros during her studies at the National Observatory for Bovine Abnormalities (www.onab.fr).

Materials

KOS microwave tissue processor (Milestone Medical)
Histological cassettes
Cassette printer
Automatic dehydration apparatus
Embedding station
Microtome
Water bath
Staining robot
Slide scanner (Pannoramic SCAN/3DHistech)
CaseViewer software

Safety warnings

Use universal safety precautions when handling samples and personal protective equipment (e.g., face mask with shield, gloves, lab coat or apron).
Xylenes and ethanol are flammable. Avoid open flames and perform procedure in a fume hood.
Gloves should be worn when performing staining process

Ethics statement

The experiments reported in this protocole comply with the ethical guidelines of the French National Research Institute for Agriculture, Food and Environment (INRAE) and its French research partners. No animal was intentionally bred for this study, and invasive sampling was performed post-mortem. Therefore, no ethical approval was required for this study. Finally, the breeders had consented to the inclusion of their animals in this study, and all the data analyzed were obtained with the permission of the breeders and breeding organizations.

Fixing the tissue

1 Fixation is the most important step in the histological preparation process. Its purpose is to preserve structures and stabilise cellular material against the potentially damaging effects of certain analytical techniques such as staining and immunohistochemistry. 1w 1d

The fixation time depends on the size of the sample. At least 10 volumes of fixative are used in relation to the size of the tissue. In our case the fixation time ranged from 48 hours to several days.

Samples are fixed in [M] 10 % volume formalin for 24:00:00 to 168:00:00 at 4 °C

Sample preparation

2 Samples are prepared differently, depending on the type of sample and what you want to highlight.
For teeth, a cross section is required to measure the depth of each component of the tooth.

3 For a bovine bone fragment (humerus, femur, incisor) measuring 2 cm x 2 cm, the following conditions were used 4h 30m

Soften the tissue by decalcifying it
Place samples in a solution of [M] 10 % volume EDTA in 1x PBS (pH 8) for 04:00:00 and 00:30:00 at 50 °C in a **KOS microwave tissue processor** (Milestone Medical)

Histology cassette mounting

4 Samples should be placed in specially designed **cassettes** to hold and flatten them. For very small samples (less than 2 mm) plastic foams are available to hold the sample in place
Cassettes should be marked with a pencil (water and alcohol resistant) or a **cassette printer**

Histology cassette

Record as many details as possible: date, project name, individual number, tissue nature, type of section

Tissue dehydration, clarification and impregnation

5 Dehydration replaces the water (80%) in the tissue with ethanol.
To do this, the cassettes are placed in an **automatic dehydration machine** which dehydrates the tissue using different alcohol solutions of increasing concentration (70% to 100%)

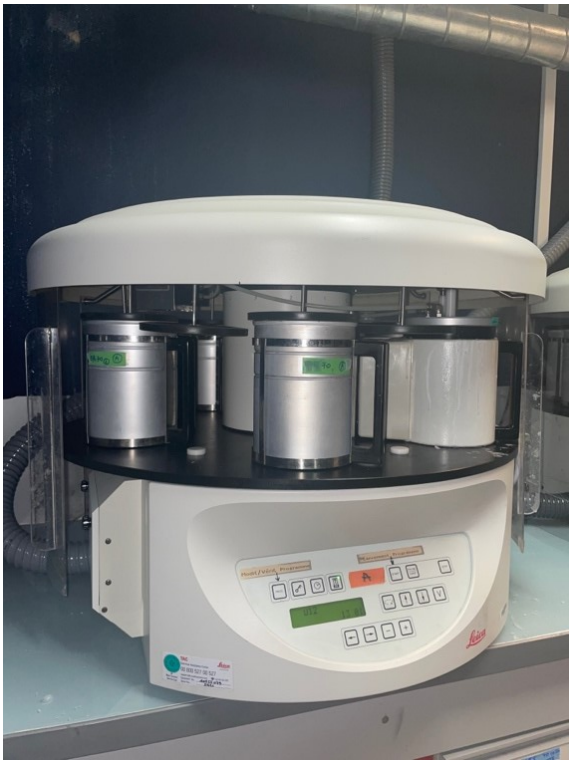
6 The machine then clarifies the samples with 2 xylene baths, removing the ethanol as paraffin is immiscible in alcohol

7 Finally, 3 paraffin baths at 60 °C (liquid) are used to impregnate the tissue. Finally, the samples are immersed in several paraffin baths.

Each step takes between 2 and 4 hours, depending on the size of the samples. For our samples, we left the fragments for 4 hours in each bath.

	A	B	C
	Steps	Bath	Time (h)
	1	70°C ethanol	4
	2	80°C ethanol	4
	3	95°C ethanol	4
	4	100°C ethanol	4
	5	Xylene	4
	6	Xylene	4
	7	Paraffin 1	4
	8	Paraffin 2	4
	9	Paraffin 3	4
		Total	36

Tissue dehydration, clarification and impregnation baths



Automatic dehydrator

Tissue embedding

8 Embedding allows the specimen to be oriented and fixed with a paraffin embedding medium so that it can be sectioned later

Place the specimen in the bottom of a metal mould

9 Place the appropriate cassette on the mould to allow identification of the tissue embedded in the paraffin block

10 Once the mould is filled, position the tissue

11 Allow the block to solidify
Wait approximately 15 minutes in the refrigerated zone until the block has stiffened and can be removed from the mould

This step can be performed using an **embedding station**

Paraffin embedding station

12 Cut the blocks into trapezoidal shapes to make it easier to cut strips to prepare the slides

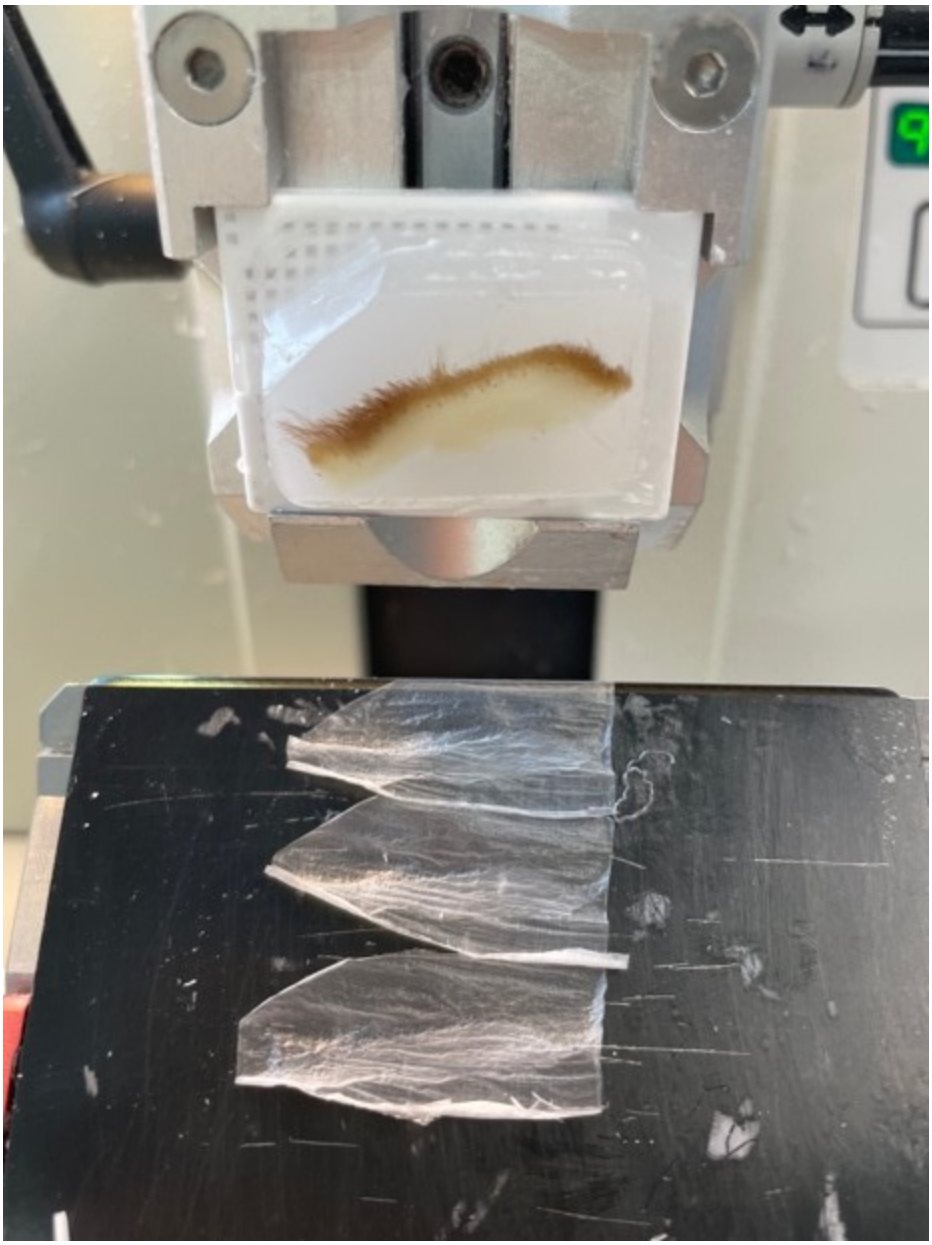
Histological sections

13 Surface of the paraffin blocks to reveal the tissue. Use a **microtome** set for a 15 micron cut

14 Slightly rehydrate the surfaced samples by placing them on damp paper Overnight at 4 °C. This will reduce the brittleness of the tissues and make them easier to cut

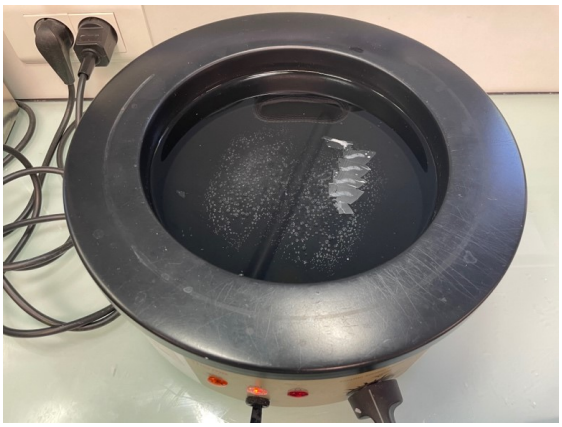
15 The samples are sectioned using an **automatically rotating microtome** (Histocore autocut). This is an important stage in the preparation of slides as it determines how well the tissue can be observed under the microscope
Make ribbons of cuts:

- Set the cut size to 5 microns
- Position the block, with the smaller side of the trapezoid facing up, as close as possible to the blade using the slider
- Unlock the blade guard
- Turn the crank clockwise
- Gently pull the ribbon with a brush to help it unwind and to avoid creasing the tissue



Example of ski cuts ribbon

16 The paraffin ribbons are placed in a **water bath** for a few seconds to allow the heat to unfold them
The ribbon is then ready to be placed on a glass slide



40°C water bath containing a ribbon

Preparation of histological slides

17 The use of adhesive slides is recommended

The slide is then dried on a slide holder in a laboratory at 40 °C for at least

Overnight

Slides, that have not been stained at this stage, are called "white" slides

White slide

Before staining, the paraffin must be removed to allow the dyes to penetrate the tissue. This step is usually included in the automated steps of a **staining robot**

Slides staining

18 Haematoxylin, eosin (HE) is a morphological usual stain. Haematoxylin is a basic dye that stains acidic structures a purplish blue, such as DNA in nuclei. Eosin is anionic and acts as an acid stain. It will therefore stain basic structures such as cytoplasm pink

Scanning and digitisation of histological slides

19 The slides are then automatically scanned and digitised using the **Pannoramic SCAN/3DHistech** tool

Pannoramic SCAN/3DHistech scanner

They can then be viewed from any computer using the dedicated **CaseViewer software** (freeware). The quality of the scanner allows colour sections to be magnified up to 63 times

Protocol references

@BRIDGe (Animal Biological Resources for Integrated and Digital Genomics) platform of the UMR GABI, Université Paris Saclay, INRAE, AgroParisTech, GABI, 78350, Jouy-en-Josas, France.