

Protocol 01	
Monitored ingredient	Bone fragments
Foodstuff	Meat products
Examination	Histochemistry, Alizarin red stainig
Short protocol/full version	Short version

1 Detection Limit

The limit of detection is not necessary define in the volume percentage, because it is ingredient which is rare in meat products. A common finding is only in case of mechanically separated meat, which includes bone fragments. Certificate of using mechanically separated meat is considered to finding 3 bone fragments in 10 histological sections.

2 Time Consumption

Sample preparation
24 hours

Sample treatment
41 minuts

3 Sample Treatment

3.1 Stainig

Sectiones are necessary before staining get rid of paraffin. Paraffin sections dissolves in the solvent (xylene) and through ethanol are the incisions converted into water because histological dyes are mostly soluble in water. We can dyed by hand in special cuvettes or in the staining automat. Dyes and dye mixtures are applied according to the procedure described below.

Alizarin red

Objective: highlighting bone fragments

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Procedure:

Getting rid of paraffin	xylene.....	10 min
	100 % alcohol + ether (2/3+1/3).....	10 min
1% acetic acid.....		Wash
Distilled water.....		Wash
Solution acid picric and alizarin.....		5 minut
Distilled water.....		Wash
Indigocarmine.....		3 min
Distilled water.....		Wash
Dewatering (alcohol)	96%.....	Wash
	100%.....	Wash
Xylene I (pure)	5 min
Xylene II (p. a.)	5 min

Preparation of Solutions:

Acid picric and alizarin:

0,5 g	alizarin red S
50 ml	distilled water
50 ml	1,2% acid picric

- Add a picric acid in dissolved alizarin in distilled water and stir. Using sodium hydroxide to adjust pH from 4.3 to 4.5. Filter and add thymol (for preservation).

Indigocarmine:

0,2 g	indigocarmine
80 ml	distilled water

Stainig results:

bone fragments	- yellow/red
fibrous connective tissue	- blue

3.2 Microscopic Examination and Evaluation of Results

The stained sections are examined by the light microscope with a lower magnification (e.g. 32x or 40x), for the study of detail is used higher magnification. Usually, only qualitative examination is performed. Describes the presence of different types of tissues in the examined

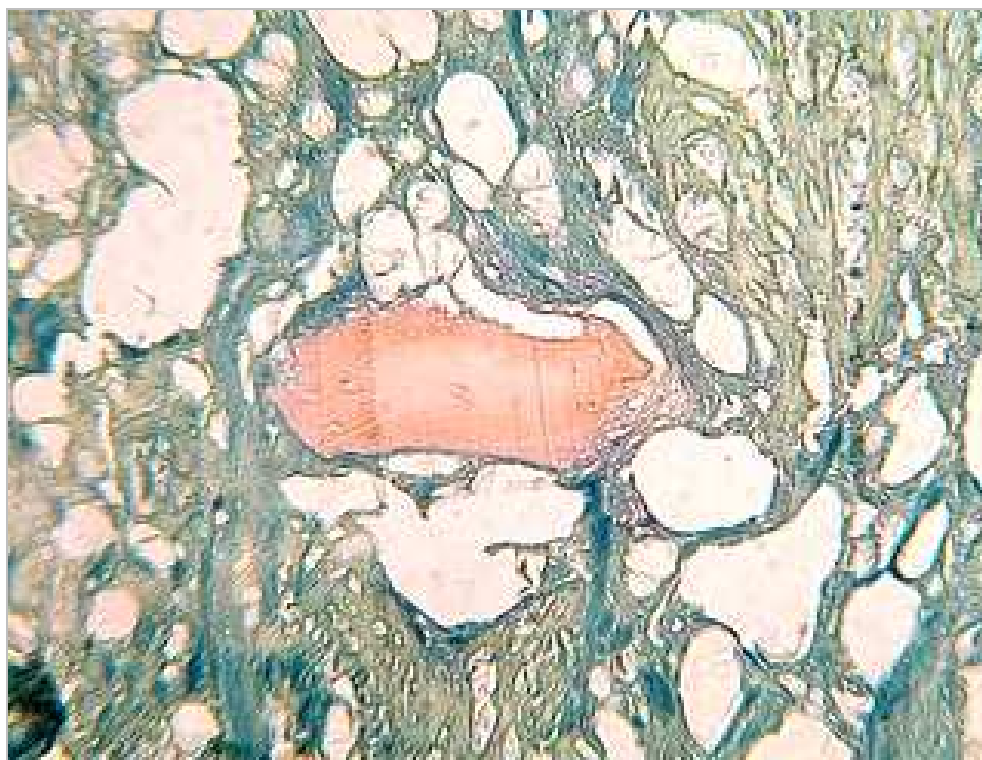
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samples. It is possible to focus only on identification of selected tissues highlighted by special staining. The identification of tissues of animal and vegetable origin must be based on data from the literature. For comparison use samples prepared in the laboratory and also the schematic pictures and photos from the literature.

3.3 Results

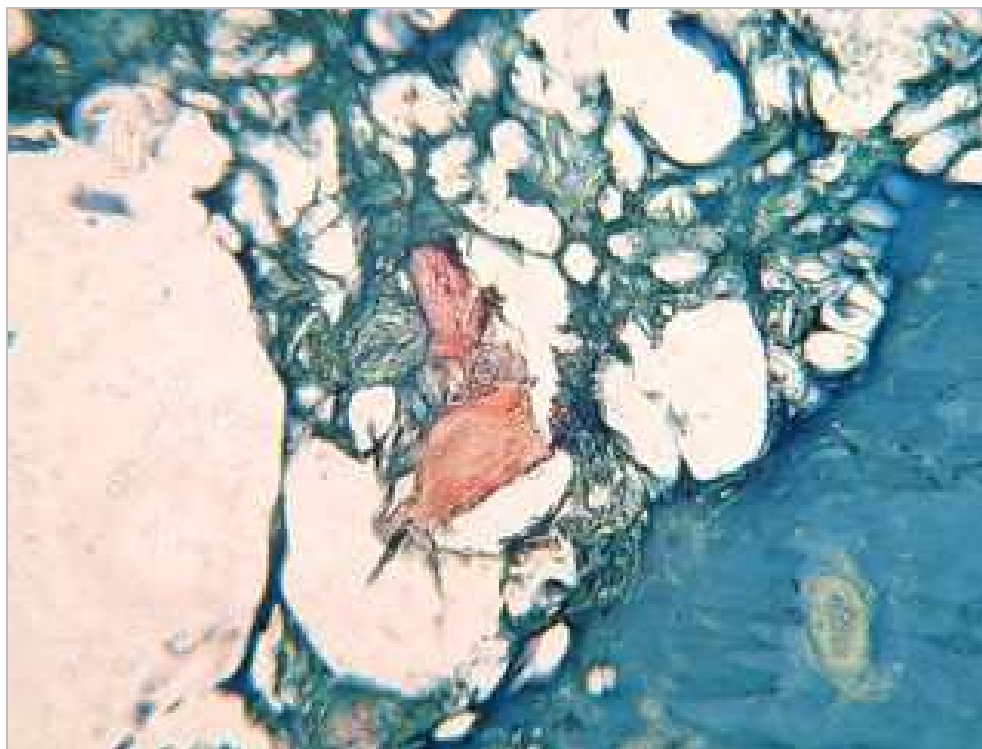
Bone fragments are stained from yellow to red.

4 Photo Documentation



Vienna sausage, stainig AC, bone fragment, magnification 250x

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Kabanos, stainig AC, bone fragment, magnification 250x

5 List of Abbreviations

AC – alizarin red