SHORT COMMUNICATION

Orcein-Picroindigocarmine—A New Multiple Stain

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Summary. A new “orcein-picroindigocarmine staining”, a colour combination of orcein, indigo carmine, and picric acid, was developed for histological applications. The new technique was tested on different human tissues.

Colours ranging from red to brown, yellow, green and blue were observed in paraffin sections of tissues stained by this method. Nuclear structures in all tissues were stained dark brown to dark blue. Squamous epithelium was stained light brown with varying shades of blue in upper horny layers, whereas the ciliated epithelium was tinged blue grey. When connective tissue was stained, collagen fibrils appeared strongly blue next to elastic fibres, which took on a rust brown tinge; cellular components were all coloured brown. The matrix of hyaline cartilage was stained in different shades of blue, with the chondrocytes rust brown. Sections of bone components appeared dark blue to dark green. Skeletal muscle cells were coloured yellow and green with blue collagenous septa.

The new staining is useful for distinguishing connective tissue components such as elastic fibres and collagen fibrils. It also demonstrates chondrocytes in favourable contrast to the cartilage matrix.

The technique produces aesthetic staining colouring that could supplement histological investigations and provide an alternative to other staining materials.

MATERIALS AND METHODS

The larynx and tongue, and skin from the upper arm were removed during autopsy, within 48 h of death, from ten bodies (five males, five females, aged 29-78 years) donated to the Department of Anatomy, Christian Albrecht University, Kiel, Germany. Limited information was available on the specimens; however, they were taken from individuals free of recent trauma or disease that might involve or affect the function of the extirpated organs and tissues.

For light microscopy the material was fixed in 4% formalin, decalcified in 20% EDTA (if necessary), dehydrated in graded concentrations of ethanol, and embedded in paraffine. Sections (7 μm) were stained with orcein, indigo carmine, and picric acid according to the following instructions: 1) deparaffinization of the tissue sections and transportation into distilled water; 2) staining with orcein (Merck, Darmstadt, Germany) for 40–50 min; 3) rinsing in distilled water; 4) differentiation in 96% alcohol, two times; 5) rinsing in distilled water; 6) staining with picroindigocarmine (0.25 g indigo carmine [Chroma, Stuttgart, Germany] in 70 ml saturated, aqueous picric acid [Merck]) for 5–10 min; 7) differentiation in 70% alcohol; 8) embedding with DePeX (Serva, Heidelberg, Germany). Finally, the stained slides were examined with a Zeiss-Axiophot microscope equipped for polarisation microscopy.

COMMENT

Multiple staining is used in histology to investigate different tissue components simultaneously. The stains used combine qualities of several individual stain characteristics, facilitating a differentiation of various cell structures and extracellular matrix components side-by-side, as practised, for example, with
van Gieson staining, azan staining and Masson’s trichrome (ROMEIS, 1989; KIERNAN, 1999). However, to show simultaneously collagen fibrils and elastic fibres in a single section, these stains have to be combined with an elastic fibre stain such as alcan blue, aldehyde fuchsin, or Weigert’s resorcin fuchsin. Alcan blue, which binds also to carboxyl groups, as found in mucosubstances, colours elastic fibres blue and can be combined with van Gieson staining which colours collagen fibrils red. Aldehyde-fuchsin also binds to carbohydrate polyacids, including heparin and the chondroitin sulphates, and stains elastic fibres purple (KIERNAN, 1999). Weigert’s resorcin-fuchsin selectively colours elastic fibres. Similar to alcan blue, Weigert’s resorcin-fuchsin cannot be used with azan or Masson trichrome staining because of their overlapping colour ranges. Combining these stains would result in both collagen fibrils and elastic fibres appearing blue, which would not allow them to be distinguished.

To develop a new, aesthetic staining technique which allows the differentiation of collagen fibrils and elastic fibres, we combined picroindigocarmine and orcein to form OPIC, the orcein-picroindigocarmine stain.

Orcein-picroindigocarmine colours tissue components as follows: nuclear structures in all tissues appear rust to dark brown; the cytoplasm of squamous epithelium cells is stained light brown, with varying shades of blue in upper horny layers (Fig. 2b); the ciliated epithelium, on the other hand, is tinted blue grey; skin appendages such as hair are stained a luminous yellow (Fig. 2f); and mucinous portions of glands are tinged light red.

In connective tissues, collagen fibrils take on a pronounced blue shade next to elastic fibres which appear rust brown. These can be examined up to the finest ramifications (Figs. 1, 2a, b, d). Connective tissue cell components such as fixed cells—fibroblasts—or mobile wandering cells—leucocytes—stain brown.

Skeletal muscle cells colour yellow to green, with blue collagenous septa. The nuclei, located at the periphery of the muscle fibres, colour red.

The matrix of hyaline cartilage stains different shades of blue. The cytoplasm of chondrocytes is rust brown or red, which surrounds a dark blue nucleus. The territorial matrix stains brown with a different intensity to that of the chondrocytic cytoplasm. The interterritorial matrix stains blue or rust brown (Fig. 2c, g, h). The tidemark is also distinguishable as a dark blue band adjacent to the subchondral bone.
Fig. 2. Legend on the opposite page.
Components of bone stain dark blue to dark green, which appear violet in polarized light microscopy (Fig. 2e).

In summary, OPIC is an alternative staining method for the examination of histological sections containing multiple tissue components. This technique demonstrates both specificity and sensitivity; in particular, collagen fibrils and elastic fibres are readily distinguished up to the finest ramifications. Hyaline cartilage is easily identified from chondrocytes and the tidemark. Based on commercial chemical prices (i.e. Merck and Fluka), OPIC-staining is an economical histological technique. It is cheaper than combinations of either azan or von Gieson staining with an elastic fibre dye. Finally, the aesthetic colouring of OPIC is an added boon that further serves to recommend the method.

REFERENCES


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