A REVIEW OF QUALITY CONTROL IN HISTOCHEMISTRY AND IMMUNOHISTOCHEMISTRY STAINING

Received: 15-07-2020 ◦ Accepted: 02-12-2020
http://dx.doi.org/10.21460/bikdw.v5i2.180

Yustina Nuke Ardiyan
Faculty of Medicine Universitas Kristen Duta Wacana, Yogyakarta, Indonesia
Correspondence: yustinanuke@staff.ukdw.ac.id

ABSTRACT

Background: Tissue staining methods need controls to give best quality results. Controls is necessary to support the validity, correctness, and reliability of the staining results. Haematoxylin and Eosin (HE) and immunohistochemistry (IHC) staining require different control quality.
Objective: To discuss the utilization of controls used in tissue staining.
Method: This is a narrative review with literature from 2008-2016.
Results: Routine staining usually does not require any control. Specific stainings require controls to support the validity, correctness, and reliability of the staining results. Histochemistry staining requires only positive control, while positive and negative controls must be included in IHC staining. A tissue section which expresses the protein of interest is reffered to positive control, whereas those without any target antigen expression are defined as negative control.
Conclusion: Histochemistry and IHC staining controls are needed to obtain the validity of the tissue or cell samples.

Keywords: immunohistochemistry, quality control, review

INTRODUCTION

There are two kinds of tissue staining methods, they are routine and specific stainings. Routine staining is used to study the microscopic structure of the cells. Hematoxylin and Eosin (HE) are basic dyes that are commonly used in this process due to their ability to differentiate nucleus from the cytoplasm. Advanced histochemistry (HC) and immunohistochemistry (IHC) spesific staining techniques are used to differentiate structure of each tissue.[1]

Histochemistry is an important staining technique that is used for the visualization of biological structures. It is used in the identification and distribution mapping of various chemical components of the tissue. The chemical components of the tissue are recognized through cell's structure identification, including cell's enzymatic activity. Some modern HC stains used are Periodic Acid Schiff (PAS), toluidine blue, and Giemsa.[2]

Immunohistochemistry staining, the utilization of antigens and antbodies interaction, uses labeled antibodies and an enzymatic reaction to identify and to localize structure of interest. There are three types of IHC staining methods: direct, indirect, and double staining. While the direct method relies on the use of a directly tagged antibody-antigen complex for detection, the indirect method make use of a labeled secondary antibody which binds to the primary antibody-antigen complex. Double staining is the indirect IHC method using two or more primary and secondary antibodies-antigen complexes. Signal amplification using enzymes and fluorescent such as avidin biotin complex (ABC) or labelled streptavidin binding (LSAB) is needed in HC staining methods to strengthen the staining sensitivity.[2]

Routine staining method requires control as a standardization of result interpretation. Control is a very important standard quality which is used as a staining reference. In advance, controls are required to ensure the correctness, validity, and reliability of staining results, especially if the sample is damaged. Based on the facts, this paper will discuss some controls which needed for both routine and specific staining.[3]

METHODS

Review of 11 articles from 2008 to 2016 is described below. This review was compiled using the narrative method, started with literature searching, literature selection based on inclusion and exclusion criteria, organizing literature, and narrating appropriate topics.

RESULTS AND DISCUSSION

Quality Control of Staining Methods

A good quality staining control is the starting point of an accurate staining method. This step must be done in every single staining process to confirm the correctness of the staining method. Control results will be used as analysis reference. Variation of tissue staining between laboratories were tested by interlaboratory comparison test with a proficiency test.[4]

Tissue and reagent controls serve the purpose of providing evidence that each staining method is succesfully performed and is giving the expected level during validation of the test for diagnostic use. Tissue control includes positive tissue control which expressed interest protein and negative tissue control which do not have any protein expresssion. Reagent control consists of no primary control, isotype control,

and absorption control. An isotype control is an antibody of the same isotype. Non-immunoreactive antibody was added in this control. Absorption controls are utilized to test if the primary antibody binds spesifically to the antigen of interest. In this control, the antibody is first incubated overnight with the immunogen. Reagent control can be used as a negative control of staining.[5] To acquire good tissue control that contains either with antigen or without antigen, tissues are placed in the same or different glass object from the sample. Same staining is performed on specimens and controls.[6]

Hematoxylin and Eosin is a routine staining commonly used. It does not require any control and the stain will not change much over the years as this stain works well with various fixatives. It provides a good illustration of nucleus, cytoplasm, and extracellular matrix. Hematoxylin stains the nucleus a purplish blue color, while Eosin gives the cytoplasm a reddish color.

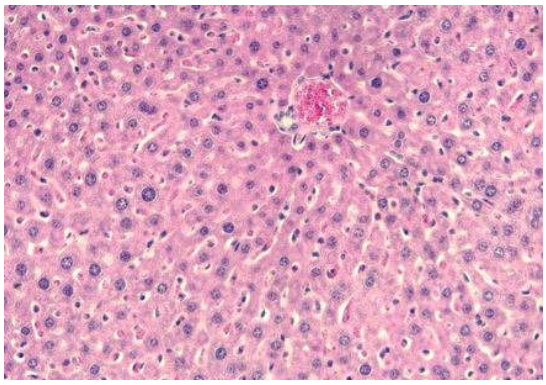


Figure 1. Liver tissue with HE staining[10]

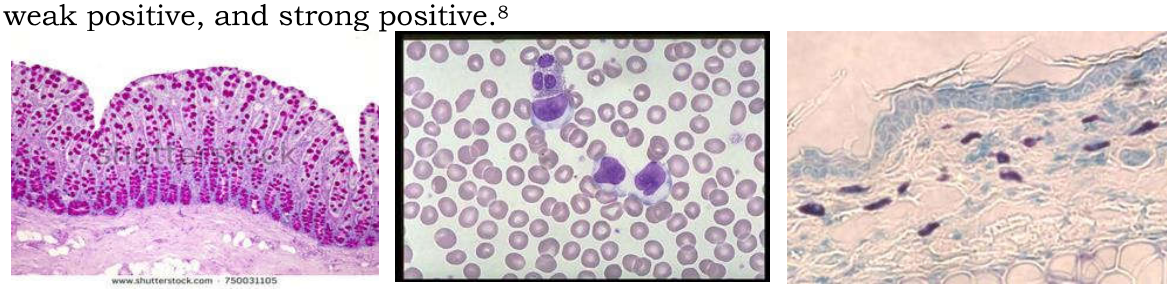
Figure 1 shows liver tissue with HE staining. In certain tissues, the nuclei are stained purple, while the cytoplasmic components and extracellular matrix have varying degrees of pink.[7]

Histochemistry staining is mainly used to distinguish certain structures by given spesific stains. Histochemistry staining usually only requires positive control, e.g., normal rat kidney to determine alkaline phosphatase activity, mouse bone tissue to exhibit calcium carbonate, normal lymph nodes to show Deoxyribo Nucleic Acid (DNA) and Ribonucleic Acid (RNA), fatty liver tissue for fat expression, and also normal intestinal tissue to reveal mucin. Other specific HC staining such as PAS is used as carbohydrate staining, it stains cell wall into red magenta or purple. Giemsa stain belongs to HC staining group, it is applied in the differentiation of blood cells and will stain red blood cells a pale pink color. Toluidine blue is a spesific stain for mast cell and it will give a violet or purple red color.[1] Some examples of tissue staining results can be seen in Figure 2.

Immunohistochemistry staining requires at least two tissue controls. Positive tissue control are specimens containing the target molecule in its know location (e.g., surface antigens, nuclear protein, and cytoplasm molecules). The histomorphology and cytomorphology of the target molecule can be visualized using fluorescence or chromogen staining. Positive control is used as antibody confirmation, whether negative control will confirm the antigen-antibody interaction in visualized reaction (Figure 3).[4]

Immunohistochemistry staining reveals various color intensities, therefore positive control with various degrees of color intensities are needed. Positive control from expected tissue of the patient's sample is known as internal positive controls, while external controls designate the use of tissues derived from souces other than the actual patient's sample. The internal positive control shows the correctness of staining process, whether external positive control provides qualitative

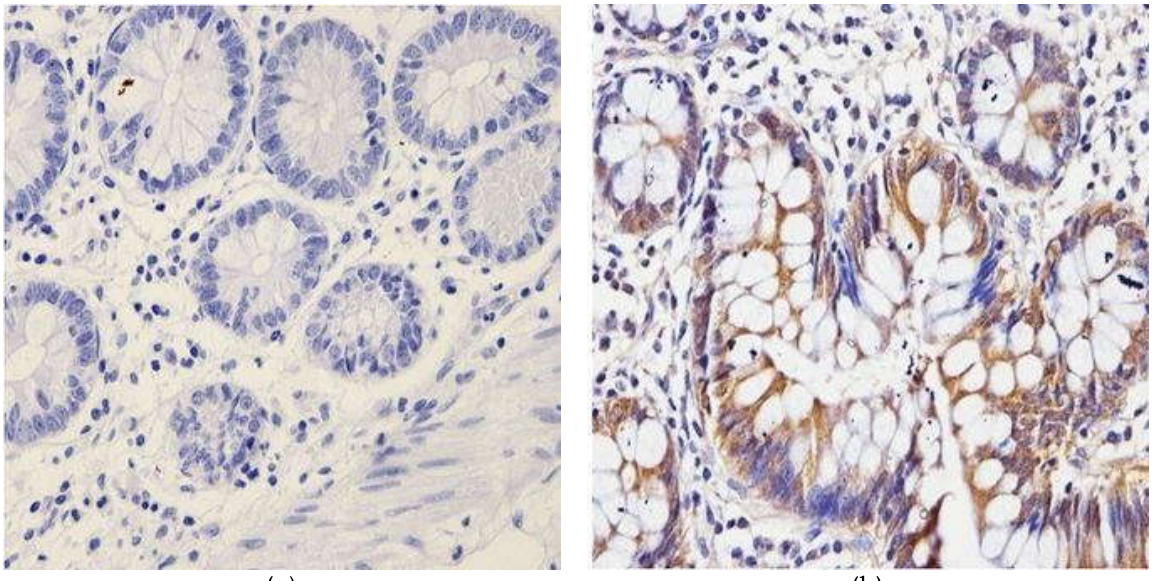
results and group them into negative, weak positive, and strong positive.[8]



(a) (b) (c)

Figure 2. (a). Colon with PAS staining, (b).Blood smear with Giemsa staining, (c).Mast cell with toluidine blue staining.[10]

Immunohistochemistry staining is widely used for diagnosis of cancers, for example breast cancer. Breast cancer diagnostic and therapeutic decision is mostly made based on the percentage of cells stained by IHC for estrogen receptor marker. One of the clinical instruments, Allred scoring system uses proportion of positive cells for estrogen receptor (score 0-5) and color intensity (score 0-3). The two parameters are summed as reference for histopathological diagnosis. Score of 0-2 is considered negative, while score 3-8 is considered positive. This scoring system uses invasive breast cancer tissue as positive control and breast tissue without primary antibody as negative control (Figure 4).[9]



(a) (b)

Figure 3. Immunohistochemistry staining, (a). Colon, negative control, (b).Colon, positive control.[10]

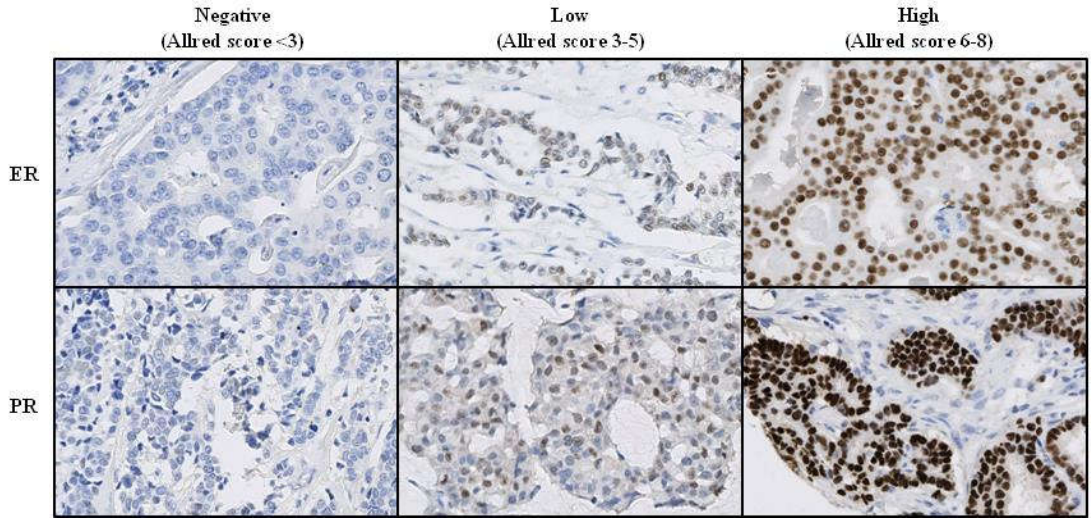


Figure 4. Immunohistochemistry staining in breast cancer with estrogen and progesterone receptore marker; negative control, weak positive (low), and strong positive (high).[11]

CONCLUSION

Histochemistry and IHC staining controls are needed to obtain the validity of the tissue or cell samples. The staining control must consist of positive and negative controls, as well as reagent controls.

ACKNOWLEDGEMENT

None

CONFLICT OF INTEREST

The author declares no conflict of interest.

REFERENCES

1. Ovalle WK, Nahirney PC. Netter's Essential Histology. 2nd ed. Philadelphia: Elsevier; 2013. Appendix, Staining methods and technique; p. 479-80.
2. Mescher AL. Junqueira's Basic Histology Text and Atlas. 12th ed. New York: McGraw-Hill Medical; 2010.
3. Otali D, Fredenburgh J, Oelschlager DK, Grizzle WE. A standard tissue as a control for histochemistry and immune-histochemistry staining. Biotech and Histochem. 2016; 91(5): 309–26.
4. Hewitt SM, Baskin DG, Frevert CW, Stahl WL, Rosa-Molinar E. Controls for immunohisto-chemistry: The histochemistry society's standards of practice for validation of immunohisto-chemistry assays. Journal of Histochemistry and Cytochemistry. 2014; 62(10): 693–7.
5. Burry RW. Controls for immunocytochemistry: An update. Journal of Histochemistry and Cyto-chemistry. 2011; 59(1): 6–12.
6. Sanderson T, Wild G, Michelle A, Jennifer C, Greg M. Immuno-histochemistry and immune-fluorescent techniques. In: Suvarna L, Christopher JD. Bancroft: Bancroft's Theory and Practice of Histological Techniques. 8th ed. Philadelphia: Elsevier, 2018:337-94.
7. Fischer AH, Jacobson KA, Rose J, Zeller R. Hematoxylin and eosin staining of tissue and cell sections. Cold Spring Harbor Protocols 2008; 3(5): 4986–8.

8. Torlakovic EE, Nielsen S, Francis G, Garrat J, Gilks B, Goldsmith JD, Hornick J, Hyjek E, Ibrahim M, Miller K, Petcu E, Swanson PE, Zhou X, Taylor CR, Vyberg M. Standardization of Negatif Kontrols in Diagnostic Immunohistochemistry: Recommendations from the International Ad Hoc Expert Panel. Appl Immunohistochem Mol Morphol. 2015; 23(1): 1–18.
9. Qureshi A, Pervez S. Allred scoring for ER reporting and its impact in clearly distinguishing ER negative from ER positive breast cancers. Journal of the Pakistan Medical Association. 2010; 60(5): 350–3.
10. H&E stains of normal tissue, special stains, immuno histochemistry stain. Available from: URL: http://www.ihcworld.com
11. Campbell EJ, Tesson M, Doogan F, Mohammed ZMA, Mallon E, Edwards J. The combined endocrine receptor in breast cancer, a novel approach to traditional hormone receptor interpretation and a better discriminator of outcome than ER and PR alone. British Journal of Cancer. 2016; 115(8): 967-973.