Difference Between Immunofluorescence and Immunohistochemistry

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Key Difference – Immunofluorescence vs Immunohistochemistry

Disease diagnostics, which uses molecular biological methods, has become an emerging area of the clinical laboratory technology. It includes all tests and methods to identify a disease and understand the cause of a disease by analyzing DNA, RNA or expressed proteins in an organism. Rapid advances in molecular diagnostics have enabled basic research on communicable and non-communicable diseases. These are used to determine changes in sequence or expression levels in crucial genes or proteins involved in disease. Immunofluorescence (IF) and Immunohistochemistry (IHC) are two such widely used techniques in cancer biology. **IF is a type of IHC where a fluorescence detection method is used to analyze monoclonal and polyclonal antibodies, whereas IHC uses chemical based methods to detect the monoclonal and polyclonal antibodies.** This is the key difference between IF and IHC.

What is Immunofluorescence (IF)?

Immunofluorescence is a detection technique where the antibodies used in the assay are labeled using fluorescent dyes or fluorescent proteins for the detection purpose. Labeled secondary antibodies can result in unwanted background signals; therefore, IF technique is based on labeling the primary antibody itself at present in order to avoid unwanted signals during detection. Through this technique, non-specific binding between the primary and the secondary antibody is prevented, and it is more rapid as there is no secondary incubation step involved. The data quality is also improved.

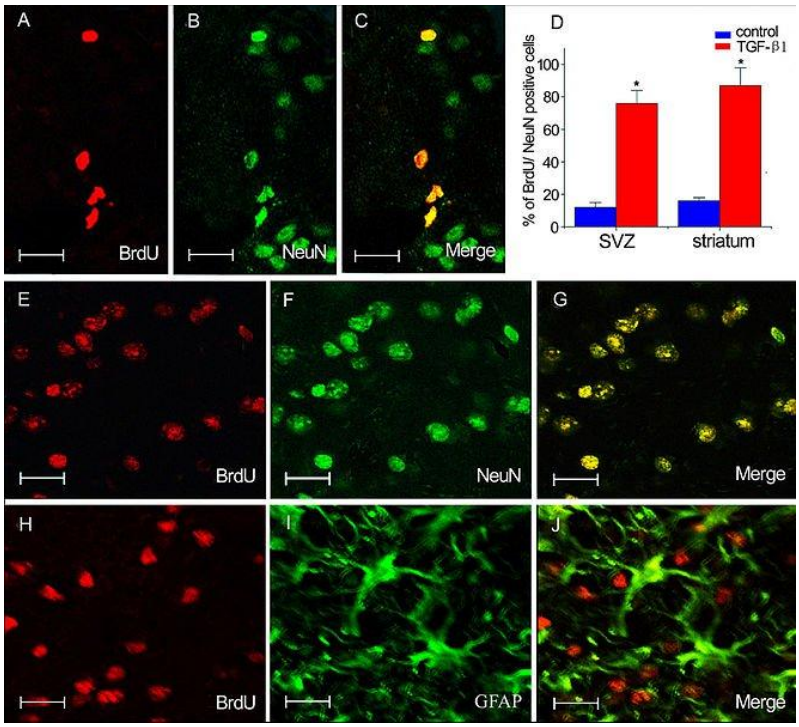

Figure 01: Double immunofluorescence staining for BrdU, NeuN, and GFAP

Fluorochromes or fluorescent dyes are compounds that can absorb radiation, preferably ultra violet radiation that is excited. When the particles reach the ground state from the excited state, they emit radiation which is captured and detected by a detector to form a spectrum. It is of great importance that the fluorescent label is compatible and stable for the particular reaction and it should be properly conjugated to the antibody in order to obtain accurate results. One of the most used fluorochromes is fluorescein isothiocyanate (FITC), which is of green color, with absorption and emission peak wavelengths of 490 nm and 520 nm, respectively. Rhodamine, another agent used in IF, is red in color and has distinct absorption and emission peak wavelengths of 553 nm and 627 nm.

What is Immunohistochemistry (IHC)?

IHC is a molecular testing method practiced in order to identify and confirm the presence of the antigen in the target cell. The target cell could be an infectious particle, a microbial pathogen or a malignant tumor cell. IHC utilizes monoclonal and polyclonal antibodies to determine the presence of antigens present on the cell surface of the target cells. The technique is based on antigen-antibody binding. A detection marker is conjugated to these antibodies in order to detect the presence or the absence of the particular antigen. These markers can be chemical markers such as enzymes, fluorescently tagged antibodies or radio labeled antibodies. The most popular

application of IHC is in cancer cell biology to identify the presence of malignant tumors, but it is also used for the detection of infectious diseases.

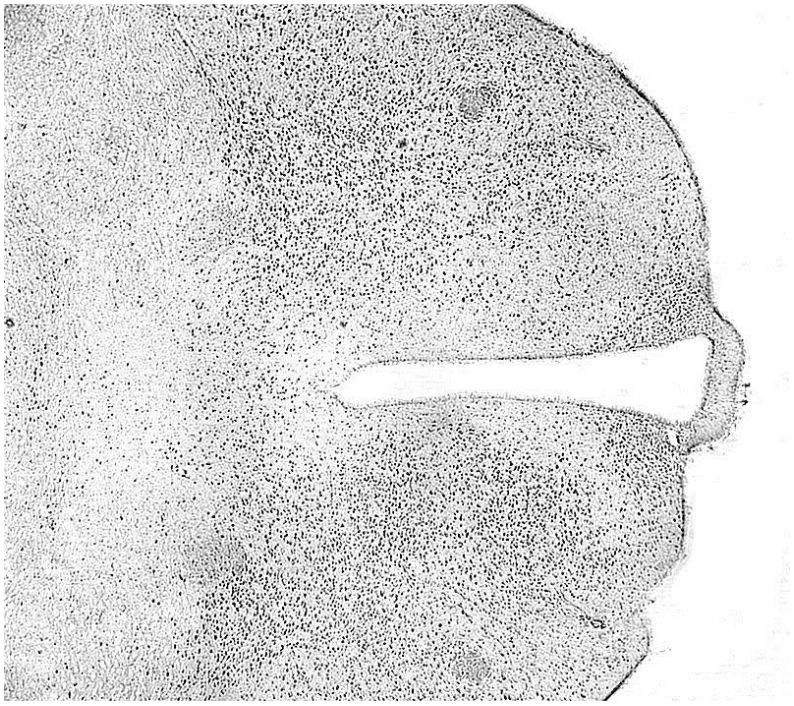

Figure 02: Mouse-brain slice stained by Immunohistochemistry

What are the similarities between Immunofluorescence and Immunohistochemistry?

- Immunofluorescence and Immunohistochemistry take place under in vitro conditions.
- Both techniques are based on the antigen-antibody
- Both are very rapid techniques.
- Results of the techniques are reproducible.
- Both have improved data quality.
- These techniques used in diagnostics for cancer and infectious diseases.

What is the difference between Immunofluorescence and Immunohistochemistry?

Immunofluorescence vs Immunohistochemistry	
IF is a detection technique where the antibodies used in the assay are labeled using fluorescent dyes or fluorescent proteins for detection.	IHC is a detection technique where the antibodies used in the assay are labeled using chemicals or radioactive elements for detection.
Accuracy	
Accuracy is higher in IF technique compared to IHC.	Accuracy is lower in IHC.
Specificity	
IF is more specific.	IHC is less specific.

Summary – Immunofluorescence vs Immunohistochemistry

Molecular mechanisms have brought about many changes in the field of medicine, giving rise to advanced molecular testing methods which have brought about revolutions in the field of diagnostics. These inventions have led to a rapid and accurate identification and confirmation of the disease, thereby enabling the successful administration and production of drugs. These techniques are also used in pharmacology in order to find the targets of drugs and to confirm the pharmacokinetic properties of the drug during drug metabolism. IF and IHC are two diagnostic methods that are based on the concept of antigen and antibody binding, although the mode of detection in both techniques differ. IF uses the principle of fluorescence to detect the antigen and IHC uses the concept of chemical conjugation to detect the antigen. This is the difference between IF and IHC.

References:

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2. Duraiyan, Jeyapradha, et al. “Applications of immunohistochemistry.” Journal of Pharmacy &Bioallied Sciences, Medknow Publications & Media Pvt Ltd, Aug. 2012, Available here. Accessed 25 Aug. 2017.

Image Courtesy:

1. “Double immunofluorescence staining for BrdU, NeuN and GFAP” By Ma M, Ma Y, Yi X, Guo R, Zhu W, Fan X, Xu G, Frey WH 2nd, Liu X. – Intranasal delivery of transforming growth factor-beta1 in mice after stroke reduces infarct volume and increases neurogenesis in the subventricular zone; PMID 19077183 (CC BY 2.0) via Commons Wikimedia
2. “Hypothalamus of a mouse tissue stained by ABC-Immunohistochemistry” By zabbn – Own work (CC BY-SA 3.0) via Commons Wikimedia

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