Letter
Volume 5:6, 2021
DOI: 10.37421/jmbp.2021.5.140

Journal of Microbiology and Pathology

Open Access

Diagnosis of Pathogenetic Microorganisms: Immunofluorescence and its limitations

Shilpa Jain*

Baylor College of Medicine, University of Houston, USA

Letter

The fluorescent antibody method is an optical microscopy using a fluorescence microscope and is mainly used for microbiological samples. This pathogenic identifying microorganism technique leverages the specificity of the antibody to the antigen to target the fluoro-chrome to a specific bio-molecular target in the cell, allowing visualization of the distribution of the target molecule throughout the sample. The specific region that an antibody recognizes on an antigen is called an epitope. Efforts have been made in epitope mapping because many antibodies can bind to the same epitope and the level of binding can differ between antibodies that recognize the same epitope. In addition, the binding of the fluorophore to the antibody itself cannot affect the immunological specificity of the antibody or its ability to bind its antigen. Immunofluorescence is a common example of immunostaining (using antibodies to stain proteins) and a specific example of immunohistochemistry (using the relationship between antibodies and antigens in tissues). This technique primarily uses a fluorophore to visualize the location of the antibody.

Immunofluorescence method can be used on tissue sections, cultured cell lines, or individual cells to analyze the distribution of proteins, glycans, and small biological and non-biological molecules. This technique can also be used to visualize structures such as medium-sized filaments. If you do not yet need to determine the topology of the cell membrane, you can use epitope insertion into the protein in combination with immunofluorescence to determine the structure. Fluorescent antibody methods can also be used as a "semi-quantitative" method for insight into DNA methylation levels and localization patterns. This is because it takes longer than a true quantitative method and there is some subjectivity in the analysis of the degree of methylation. Immunofluorescence can be used in combination with fluorescent staining of other non-antibody methods, such as the use of DAPI to label DNA. Various microscope designs can be used to analyze fluorescent antibody samples. The simplest is the epi-fluorescence microscope, and confocal microscopes are also widely used. Various super-resolution microscopy designs are also available that allow for much higher resolution.

Like most fluorescence techniques, photo bleaching is a major problem in immunofluorescence. Loss of activity caused by photo bleaching reduces or limits the intensity or duration of exposure, increases the concentration of fluorophore, or is more robust fluorophore that is less prone to fading (eg Alexa Fluors, Seta Fluors or It can be controlled by using DyLight-Fluores). Some of the problems that can arise from this approach include autofluorescence, unrelated unnecessary specific fluorescence, and non-specific fluorescence. Autofluorescence includes fluorescence emitted from the sample tissue or the cells themselves. If the target antigen is impure and contains antigen contaminants, extraneous unwanted specific fluorescence will occur. Non-specific fluorescence includes loss of probe specificity due to fluorophore, improper fixation, or sample drying.

Immunofluorescence is limited to fixed (ie, dead) cells when visualizing intracellular structures. This is because the antibody does not penetrate the cell membrane when it reacts with the fluorescent marker. Antigenic substances need to be firmly anchored in their natural position within the cell. Intact antibodies can also be too large to stain cancer cells in vivo. Their size slows tumor penetration and has a long circulating half-life. Studies are underway to investigate the use of dia body to avoid this limitation. Proteins outside the supernatant or cell membrane can be bound by antibodies. This allows you to stain living cells. Depending on the fixation solution used, the protein of interest can be cross-linked. This can cause false positive or false negative signals due to non-specific binding.

Another approach is to use recombinant proteins that contain fluorescent protein domains. B. Green fluorescent protein (GFP). The use of such "labeled" proteins allows their localization to be determined in living cells. Even if this appears to be a sophisticated alternative to immunofluorescence, cells should be transfected or transduced with GFP tags. This will result in organisms of at least S1 and above, requiring stricter safety standards in the laboratory. This technique modifies the genetic information of cells.

***Address for Correspondence:** Shilpa Jain, Baylor College of Medicine, University of Houston, USA, E-mail: shilpajain@gmail.com*

Received 04 November 2021; **Accepted** 11 November 2021; **Published** 18 November 2021

How to cite this article: Shilpa Jain. "Diagnosis of Pathogenetic Microorganisms: Immunofluorescence and its limitations." *J Microbiol Pathol* 5 (2021): 140.