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An Overview of Special Techniques on Immunohistochemistry and *In Situ* Hybridization

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Description

Histopathologic tests improve with molecular assays because they increase specificity and, in some cases, sensitivity. When microorganisms are undetected by histochemical approaches, are present in low quantities, stain poorly, are uncultivable, or have an unusual morphology, molecular methods of identification may be very beneficial. Molecular approaches are useful for the quick, specific, and quantitative identification of microbes in various situations. Following the use of typical histochemical stains, they are the next stage in histopathologic testing for the detection of infectious agents.

Immunohistochemistry has revolutionised histopathology, particularly when it comes to classifying solid tumours and haematological neoplasms, as well as identifying infectious agents. It is the most often used auxiliary diagnostic tool for the detection of microorganisms in histologic sections after histochemical staining. Monoclonal or polyclonal antibodies directed against specific microbial antigens are used in this method. The antibodies are detected by either fluorescent or chromogenic signal amplification once they have been bound. The specificity of this approach is determined by the specificity of the immunoglobulin molecule's antigen binding (Fab) region. Fresh, frozen tissue is used for immunofluorescent immunohistochemistry, whereas formalin-fixed, paraffin-embedded tissues are used for immunoperoxidase techniques.

These methods can be used to detect fastidious or noncultivable microbes, distinguish between morphologically identical microorganisms or cytopathic effects, and detect highly infectious microorganisms that are engaged in infection outbreaks. Fastidious microorganisms are particularly crucial to detect using supplementary approaches since they may go undetected in the microbiology laboratory. *Rickettsia*, the causal agent of Rocky Mountain spotted fever, for example, is rarely cultivated, but it can be easily discovered in biopsies of infected patients' skin samples using immunofluorescence or immunoperoxidase methods.

Immunohistochemical stains have been utilised to distinguish morphologically similar microorganisms such as *Histoplasma, Trypanosoma, and Leishmania* species, and such strains have been created as an aid to Chagas' disease histopathologic diagnosis. These immunohistochemical approaches have also been used to discriminate morphologically similar cytopathic effects, such as those caused by HSV and VZV. Immunohistochemistry may also be more sensitive than histologic sections for detecting bacteria that are difficult to find. Indeed, automation may make this more cost-effective than manual histochemical approaches.

Finally, when studying tissues from individuals engaged in infectious outbreaks, immunohistochemistry approaches may be informative. Given the risk to laboratory personnel who handle live virus in outbreak situations involving a highly infectious agent with a high mortality rate, immunohistochemical examination of formalin-fixed, noninfectious tissues would be preferred to culture in outbreak situations involving a highly infectious agent with a high mortality rate. These approaches, for example, were utilised to document patients during a leptospirosis epidemic in Nicaragua in 1998 and to detect the Ebola virus. Antibodies required to detect agents of exotic infectious illnesses are rarely commercially accessible, but they are utilised in tests that are conducted by the Centers for Disease Control and Prevention, the National Center for Infectious Diseases, and other specialist laboratories.

Many of the advantages of in situ hybridization are similar to those of immunohistochemistry. Instead of using an antibody, this approach exploits the complementary nature of nucleic acids to confer specificity. The nucleic acid probe anneals to a specific target sequence in microbial DNA or RNA, which can be tagged in a variety of ways. Methods comparable to those employed in immunohistochemistry are used to generate a signal. In situ hybridization is growing increasingly popular, and with the development of automated and standardised procedures, it is becoming more readily available and less expensive.