

PRINCIPLES FOR OPTIMIZING CLINICAL AND LABORATORY DIAGNOSTICS OF INFECTIOUS DISEASES

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Introduction. A bacterium, virus, fungus, protozoan, or helminth found in a patient with a suitable clinical condition is used to diagnosis infectious disorders. The detection techniques include isolating viruses in cell culture, growing bacteria and fungus on growth media, and identifying the agent biochemically, antigenically, or genetically. Additionally, a particular immune response—typically antibodies—that arises during the course of sickness can be used to identify infectious disorders. When an agent is seen in infected tissue, a diagnosis can be made based on certain morphological traits or the type of organism, such as gram-positive or gram-negative bacteria or viruses (e.g., eosinophilic cytoplasmic inclusion bodies in neurons in rabies virus infection). More precise diagnoses are made using techniques that identify and visualize antigens (immunohistochemistry) or nucleic acid sequences (in situ hybridization). One effective molecular diagnostic method is the detection of particular nucleic acid sequences amplified by polymerase chain reaction. Since the first infectious disease, anthrax, was identified more than 135 years ago, new technology and agents have led to the application and modification of Koch's postulates. Over 70 hitherto unidentified infection agents have been found in newly emerging infectious diseases over the past 45 years, and this trend is probably going to continue [1,2,3,4].

Material and methods. Identifying an infection in situ. The actual quantity of pathogens in tissues is often unexpectedly low because host immune mechanisms can significantly increase the host response. This implies that before a pathogen is found, a number of areas may need to be looked at. It is not unusual for a pathologist to look at just one histochemically stained tissue section when diagnosing an infection, even though few surgical pathologists would object to the idea of ordering more sections to rule out malignancy in a biopsy they considered suspicious. The idea that the microbiology lab will eventually identify the causal infectious agent, negating the necessity for the surgical pathologist to complicate the procedure, is even more heinous. **Possible Restrictions on Biopsy Interpretation.** The sensitivity and specificity of histochemically stained sections are limited in certain circumstances, notwithstanding the benefits of using biopsy specimens for infection diagnosis. For instance, in nearly half of cases of tuberculosis, biopsies may not reveal mycobacteria. Even so, the onset of the inflammatory response in situ should support a workable diagnosis that is frequently trustworthy enough to start practical treatment [1,2,3].

Results and discussion. Another method for identifying infectious organisms in formalin-fixed, paraffin-embedded tissue is the branch chain in situ hybridization (bISH) assay. Unlike PCR, this method does not amplify the target; instead, it amplifies the signal using a branching DNA structure. Although it is much lower than PCR-based methods, the signal amplification dramatically raises the assay's sensitivity. The assay's sensitivity is significantly increased by hybridizing many tiling probes for a gene to the target RNA, which are usually dispersed throughout the genome of interest. A sequence of hybridization processes results in the creation of a signal amplification structure. The branching structure that results from the hybridization of several alkaline

phosphatase-conjugated label probes has about 400 binding sites for the alkaline phosphatase-labeled probe, which may be seen as a red chromogen under a bright field microscope. Usually, the signal appears as dots, but when transcripts are strongly expressed, individual dots are no longer visible. These tests can be carried out using clinical-grade automated immunohistochemistry equipment and are commercially available [1,2,3,4].

Conclusions. The pathology and pathophysiology of Lassa fever, Rocky Mountain spotted fever, Mediterranean spotted fever, and human monocytotropic ehrlichiosis have all been clarified by his research, which has also produced animal models for studying rickettsioses and ehrlichioses. *Anaplasma phagocytophilum* (human granulocytotropic anaplasmosis), *Rickettsia japonica* (Japanese spotted fever), *Rickettsia felis* (flea-borne spotted fever), *E. chaffeensis* (human monocytotropic ehrlichiosis), and severe fever and thrombocytopenia syndrome virus were among the emerging infections that he helped discover, characterize, and/or epidemiology. It is important to understand the bISH RNA assay's limitations. Even though nonspecific staining is often less than one dot per ten cells, it can be much higher, making it impossible to tell the difference between a true and nonspecific signal. Rapid formalin fixation is frequently required for the best preservation of RNA, and poor RNA quality could compromise the experiment. Lastly, the majority of successful clinical-grade bISH assays express several hundred target RNA transcripts per cell, despite the assay's theoretical ability to detect a single transcript.

Adabiyotlar, References, Литературы:

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