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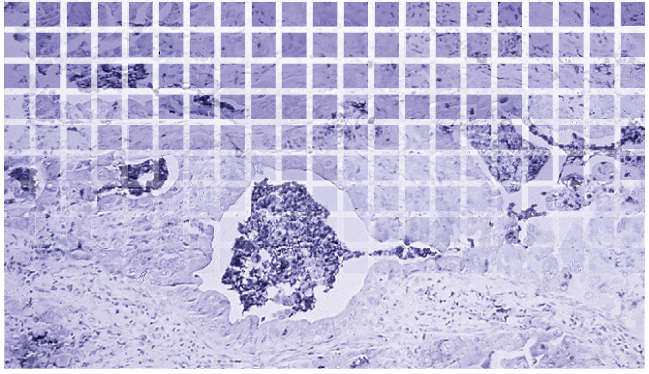

3

Immunohistology of Infectious Diseases

Eduardo J. Ezyaguirre • David H. Walker • Sherif R. Zaki

INTRODUCTION

Since the 1980s, immunohistochemistry (IHC) has dramatically transformed the approach to histopathologic diagnosis, specifically in the diagnosis and classification of tumors, and more recently in the diagnosis of infectious diseases in tissue samples.[1]

Pathologists play an important role in recognizing infectious agents in tissue samples from patients, providing a rapid morphologic diagnosis, and facilitating clinical decisions in patient treatment. When fresh tissue is not available for culture, pathologists can provide a rapid morphologic diagnosis and facilitate clinical decisions in patient treatment.[2] In addition, pathologists have played a central role in identifying emerging and reemerging infectious agents, describing the pathogenetic processes of emerging diseases (e.g., hantavirus pulmonary syndrome, viral hemorrhagic fevers, leptospirosis, and rickettsial and ehrlichial infections), and diagnosing anthrax during the bioterrorist attack of 2001.[3-7]

Cultures and serologic assays are usually used for microbial identification in infectious diseases. However, fresh tissue is not always available, and culturing fastidious pathogens can be difficult and may take weeks or months to yield results. Moreover, culture alone cannot distinguish colonization from tissue invasion. In addition, serologic results can be difficult to interpret in the setting of immunosuppression or when only a single sample is available for evaluation. Some microorganisms have distinctive morphologic characteristics that allow their identification in formalin-fixed tissues using routine and special stains. Nevertheless, in many instances it is difficult or even impossible to identify an infectious agent specifically by conventional morphologic methods.

Immunohistochemistry is one of the most powerful techniques in surgical pathology. There has been an increasing interest in the use of specific antibodies to viral, bacterial, fungal, and parasitic antigens in the detection and identification of the causative agents in many infectious diseases. Coons and associates were the first to use a specific antibody to detect a microbial antigen to detect pneumococcal antigen in tissues.[8] The advantages of IHC over conventional staining methods (Table 3.1) and the contributions of IHC in infectious diseases (Table 3.2) are substantial. In many instances, IHC has shown high specificity, allowing the differentiation of morphologically similar microorganisms.[9] Immunohistochemistry is especially useful when microorganisms are difficult to identify by routine or special stains, are fastidious to grow, or exhibit atypical morphology (Table 3.3).[10-14] It is important to understand that there may be widespread occurrence of common antigens among bacteria and pathogenic fungi, and both monoclonal and polyclonal antibodies must be tested for possible cross-reactivity with other organisms.[15] Finally, it is important to emphasize that IHC has several steps, and that all of them can affect the final result; however, in general the only limitations are the availability of specific antibodies and the preservation of epitopes.[16]

Table 3.4 lists some commercially available antibodies for diagnostic use in surgical pathology.

TABLE 3.1	Advantages of IHC for the Diagnosis of Infectious Diseases
1.	Opportunity for rapid results
2.	Reduced risk of exposure to serious infectious diseases by performance on formalin-fixed, paraffin-embedded tissue
3.	High sensitivity allowing identification of infectious agents even before morphologic changes occur
4.	Opportunities for retrospective diagnosis of individual patients and for in-depth study of the disease
5.	Specific identification of infectious agents with many monoclonal antibodies and some polyclonal antibodies

TABLE 3.2	Contributions of IHC to the Diagnosis of Infectious Diseases
1.	Allows identification of new human pathogens
2.	Allows microbiological-morphological correlation establishing the pathogenic significance of microbiological results
3.	Provides a rapid morphologic diagnosis allowing early treatment of serious infectious diseases
4.	Contributes to understanding of the pathogenesis of infectious diseases
5.	Provides a diagnosis when fresh tissue is not available or when culture methods do not exist

TABLE 3.3	Applications of IHC in the Diagnosis of Infectious Diseases
1.	Identification of microorganisms that are difficult to detect by routine or special stains
2.	Detection of microorganisms that are present in low numbers
3.	Detection of microorganisms that stain poorly
4.	Identification of microorganisms that are fastidious to grow or noncultivable
5.	Identification of microorganisms that exhibit atypical morphology

VIRAL INFECTIONS

Immunohistochemistry has played an important role not only in the diagnosis of a large number of viral infections but also in the study of their pathogenesis and epidemiology. Conventionally, the diagnosis of viral infections has relied on cytopathic changes observed by routine histopathologic examination. Several viral pathogens produce characteristic intracellular inclusions, which allow pathologists to make a presumptive diagnosis of viral infection. However, for some viral infections the characteristic cytopathic changes are subtle and sparse, requiring a meticulous search.[17] Moreover, only 50% of known viral diseases are associated with characteristic intracellular inclusions.[18] In addition, formalin, which is the most commonly used fixative in histopathology, is a poor fixative for demonstrating the morphologic and tinctorial features of viral inclusions.[19] When viral inclusions are not detected in hematoxylin and eosin–stained sections or when the viral inclusions present cannot be differentiated from those of other viral diseases, immunohistochemical techniques offer a more reliable approach to reach a specific diagnosis.

Hepatitis B

Hepatitis B virus infection constitutes an important cause of chronic hepatitis in a significant proportion of patients. In many instances, the morphologic changes induced by hepatitis B virus in hepatocytes are not typical enough to render a presumptive diagnosis of hepatitis B viral infection. In other instances, there may be so little hepatitis B surface antigen (HBsAg) that it cannot be demonstrated by techniques such as orcein staining. In these cases, immunohistochemical techniques to detect HBsAg are more sensitive than histochemical methods and are helpful in reaching a diagnosis.[20] Immunostaining for HBsAg has been used in the diagnosis of hepatitis B and in the study of carrier states.[21,22] Eighty percent or more of cases with positive serologic results for HBsAg demonstrate cytoplasmic HBsAg using IHC.[23] By immunoperoxidase localization, hepatitis B core antigen (HBcAg) can be demonstrated within the nuclei or the cytoplasm of hepatocytes, or both. Cytoplasmic expression of HBcAg usually is associated with a higher grade of hepatitis activity,[23] and diffuse immunostaining of nuclei for HBcAg generally suggests uncontrolled viral replication in the setting of immunosuppression.[24] Immunostaining for HBsAg and HBcAg is useful in the diagnosis of recurrent hepatitis B infection in liver allografts, particularly when present with atypical histopathologic features.[25]

Herpesviruses

Histologically, the diagnosis of herpes simplex virus (HSV) infection involves the detection of multinucleated giant cells containing characteristic molded, ground glass–appearing nuclei and Cowdry's type A intranuclear inclusions. When abundant viral inclusions exist within infected cells, the diagnosis is usually straightforward. However, diagnosis can be difficult when the characteristic intranuclear inclusions or multinucleated cells, or both, are absent or when the amount of tissue in a biopsy specimen is small.[26] In these cases, IHC using either polyclonal or monoclonal antibodies against HSV antigens has proven to be a sensitive and specific technique used to diagnose HSV infections (Fig. 3.1).[27-30]

Although polyclonal antibodies against major HSV glycoprotein antigens are sensitive, they do not allow distinction between HSV-1 and HSV-2; this is because the two viruses are antigenically similar.[31] In addition, the histologic features of HSV infection are not specific and can also occur in patients with varicella-zoster (VZV) infection. Monoclonal antibodies against the VZV envelope glycoprotein gp1 are sufficiently sensitive

TABLE 3.4 Commercially Available Antibodies for Immunohistochemical Diagnosis of Infectious and Prion Diseases

Microorganism	Antibody/Clone	Dilution	Pretreatment	Source
Adenovirus	Mab/20/11 and 2/6	1:2000	Proteinase K	Chemicon
B. henselae	Mab	1:100	HIAR	Biocare Medical
BK virus	Mab/BK T.1	1:8000	Trypsin	Chemicon
C. albicans	Mab/1B12	1:400	HIAR	Chemicon
C. pneumoniae	Mab/RR402	1:200	HIAR	Accurate
Cryptosporidium	Mab/Mabc1	1:100	HIAR	Novocastra
CMV	Mab/DDG9/CCH2	1:50	HIAR	Novocastra
Clostridium spp.	Rabbit polyclonal	1:1000	None	Biodesign
G. intestinalis	Mab/9D5.3.1	1:50	HIAR	Novocastra
Hepatitis B core antigen	Rabbit polyclonal	1:2000	HIAR	Dako
Hepatitis B surface antigen	Mab/3E7	1:100	HIAR	Dako
Herpes simplex 1 and 2 viruses	Rabbit polyclonal	1:3200	HIAR	Dako
H. pylori	Rabbit polyclonal	1:40	Proteinase K	Dako
HHV 8	Mab/LNA-1	1:500	HIAR	Novocastra
K. pneumoniae	Rabbit polyclonal	1:200	Proteinase K	Biogenex
L. monocytogenes	Rabbit polyclonal	1:5000	Proteinase K	Difco
M. pneumoniae	Mab/1.B.432	1:25	HIAR	US Biological
Parvovirus B19	Mab/R92F6	1:500	HIAR	Novocastra
P. carinii	Mab/3F6	1:20	HIAR	Novocastra
P. falciparum	Mab/BDI400	1:1000	Proteinase K	Biodesign
Prion	Mab/3F4	1:200	Antigen retrieval	Dako
	Mab/12F10	1:1000	Proteinase K	Cayman Chemical
	Mab/KG9	1:1000	Proteinase K	TSE Resource Center
Respiratory syncytial virus	Mab/5H5N	1:200	HIAR	Novocastra
S. aureus	Rabbit polyclonal	1:500	Proteinase K	Biodesign
T. pallidum	Rabbit polyclonal		HIAR	Biodesign
T. gondii	Rabbit polyclonal	1:320	HIAR	Biogenex
West Nile virus	Mab/5H10	1:400	Proteinase K	Bioreliance

and specific to allow a clear-cut distinction between HSV and VZV infections.[27,32,33]

Immunohistochemistry has also been useful in demonstrating the association of human herpes virus 8 (HHV-8) with Kaposi's sarcoma, primary effusion lymphoma, and multicentric Castleman's disease.[34-38] Diagnosis of Kaposi's sarcoma may be problematic because of its broad morphologic spectrum and similar appearance to other benign and malignant neoplastic vascular lesions. Immunostaining of latent associated nuclear antigen-1 (LANA-1) is useful to confirm the diagnosis of Kaposi's sarcoma, particularly when difficult early lesions closely resemble the appearance of interstitial granuloma annulare and when the neoplasm presents in an unusual location. Immunostaining also allows distinction of Kaposi's sarcoma from several morphologically similar vasoproliferative lesions.[39-41] Immunostaining is restricted to the nuclei of spindle cells and endothelial cells of the slitlike vascular spaces (Fig. 3.2). Immunohistochemistry has also demonstrated expression of HHV-8 LANA-1 in mesothelial cells of HIV-associated recurrent pleural effusions.[42]

Cytomegalovirus (CMV) continues to be an important opportunistic pathogen in immunocompromised patients; it is estimated that 30% of transplant recipients experience CMV disease.[43] The range of organ involvement in post-transplant CMV disease is wide; hepatitis occurs in 40% of liver transplant recipients,[44] and pneumonitis is more frequently seen in heart and heart-lung transplant patients.[45] Other organs that are commonly affected are the gastrointestinal tract and the peripheral and central nervous systems. Histologic diagnosis of CMV in fixed tissues usually rests on identifying characteristic cytopathic effects including intranuclear inclusions, cytoplasmic inclusions, or both. However, histologic examination lacks sensitivity, and in some

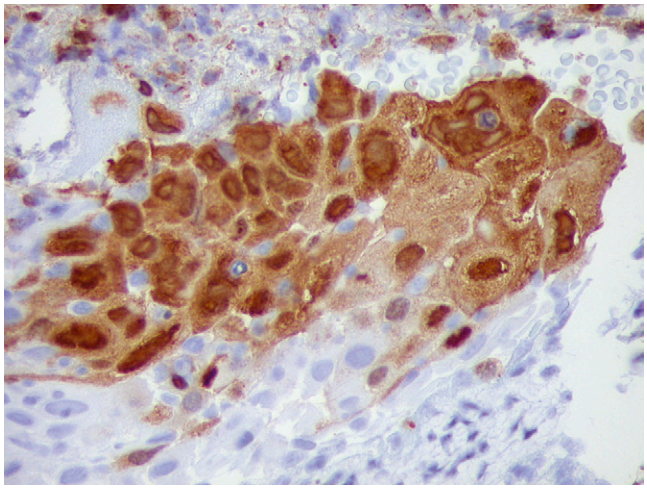

FIGURE 3.1 Photomicrograph of cervical biopsy from a patient with herpes simplex virus infection showing abundant nuclear and cytoplasmic antigen. (Immunoperoxidase staining with DAB and hematoxylin counterstain; ×400.)

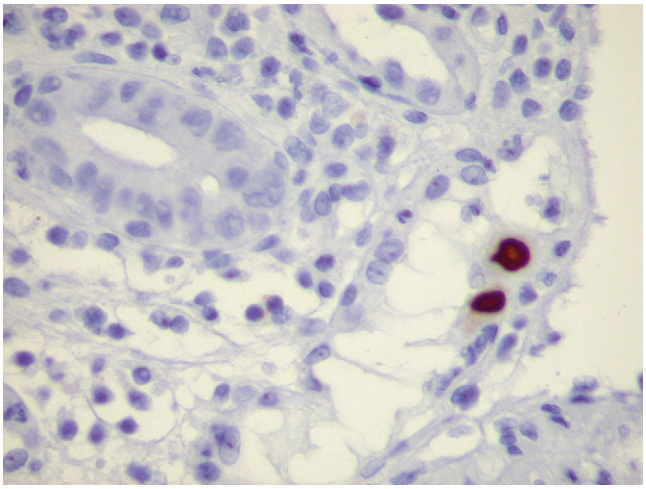

FIGURE 3.3 Colon biopsy of a patient with steroid-refractory ulcerative colitis. Rare epithelial cells show intranuclear CMV antigen. (Immunoperoxidase staining with DAB and hematoxylin counterstain; ×400.)

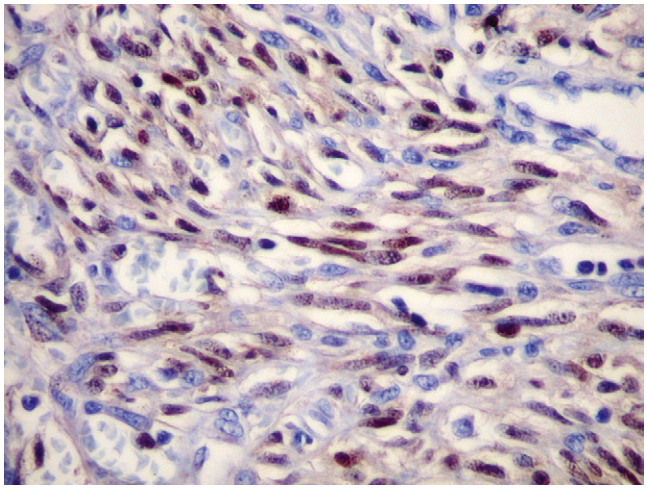

FIGURE 3.2 Lymph node biopsy from a patient with Kaposi's sarcoma. The spindle cells show strong nuclear staining for HHV-8 LANA-1 antigen. Endothelial cells of well formed vascular spaces are negative. (Immunoperoxidase staining with DAB and hematoxylin counterstain; ×400.)

cases atypical cytopathic features can be confused with reactive or degenerative changes.[46] Additionally, up to 38% of patients with gastrointestinal CMV disease fail to demonstrate any inclusions.[47] In these cases, IHC using monoclonal antibodies against early and late CMV antigens allows the detection of CMV antigens in the nucleus and cytoplasm of infected cells (Fig. 3.3). The sensitivity of IHC for detecting CMV infection ranges from 78% to 93%.[47,48] In addition, IHC may allow detection of CMV antigens early in the course of the disease when cytopathic changes have not yet developed.[49-54] For example, CMV early nuclear antigen is expressed 9 to 96 hours after cellular infection and indicates early active viral replication. Immunohistochemistry has been used to detect CMV infection in patients with steroid refractory ulcerative colitis, and the routine use of IHC for the detection of CMV in the evaluation of these patients is now recommended.[55,56] CMV immunostaining has been used to detect occult CMV infection of the central nervous system in liver transplant patients who develop neurologic complications.[57] It has also been used to demonstrate a high frequency of CMV antigens in tissues from first-trimester abortions.[58] CMV is the most common opportunistic organism found in liver biopsies from transplant patients; nonetheless, the incidence of CMV hepatitis appears to be decreasing owing to better prophylactic treatments.[59] Although CMV hepatitis presents with characteristic neutrophilic aggregates within the liver parenchyma, atypical features suggestive of acute rejection or changes indistinguishable from those of any other viral hepatitis are occasionally observed.[60] In addition, parenchymal neutrophilic microabscesses have been described in cases with no evidence of CMV infection.[61] In these cases, immunostaining for CMV antigens is most useful in determining the diagnosis of CMV infection.[62]

The sensitivity of IHC is better than light microscopic identification of viral inclusions and compares favorably with culture and *in situ* hybridization.[49,51,52,54,63] Additionally, immunohistochemical assays can be completed faster than the shell vial culture technique, allowing for rapid results that are important for early anti-CMV therapy.[54]

Other herpesvirus infections that have been diagnosed using immunohistochemical methods include human herpesvirus 6 infection[64] and Epstein-Barr viral infection.[65] Immunohistochemistry has been used to identify EBV latent membrane protein-1 in cases of Hodgkin's lymphoma and post-transplant lymphoproliferative disorder (Fig. 3.4).[66]

Adenoviruses

Adenovirus has been increasingly recognized as a cause of morbidity and mortality among immunocompromised patients owing to transplant and congenital immunodeficiency.[67,68] Rarely adenovirus infection has been described in HIV-infected patients.[69-71] Characteristic

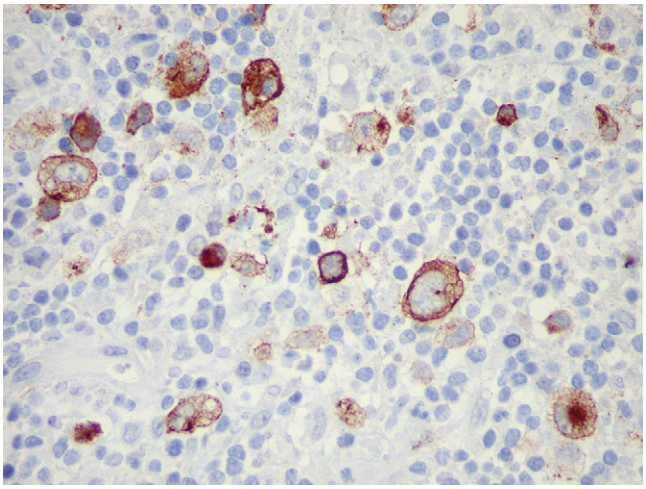

FIGURE 3.4 Epstein-Barr virus LMP-1 within cytoplasm of characteristic Reed-Sternberg cells in a case of Hodgkin's lymphoma. (Immunoperoxidase staining with DAB and hematoxylin counterstain; ×400.)

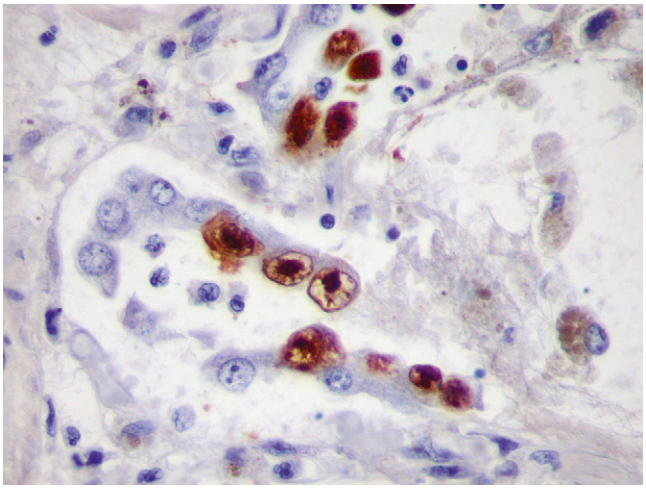

FIGURE 3.5 Adenovirus pneumonia in a heart transplant patient who developed ARDS and respiratory failure. Infected cells within necrotizing exudate show intranuclear reactivity with antibody to adenovirus antigen. Some cells show inclusions with a clear halo around them, making a differential diagnosis from CMV difficult on H&E stain. (Immunoperoxidase staining with DAB and hematoxylin counterstain; ×400.)

adenovirus inclusions are amphophilic, intranuclear, homogeneous, and glassy. However, in some cases, the infection may contain only rare cells showing the characteristic cytopathic effect.[70] In addition, other viral inclusions, including CMV, human papillomavirus (HPV), HSV, and VZV, can be mistaken for adenovirus inclusions and vice versa. In these circumstances, immunohistochemical assay may be necessary for a definitive diagnosis. A monoclonal antibody that is reactive with all 41 serotypes of adenovirus has been used in an immunohistochemical technique to demonstrate intranuclear adenoviral antigen in immunocompromised patients (Fig. 3.5).[70-74] Histologic diagnosis of adenovirus colitis is difficult, and it is usually underdiagnosed. Moreover, in immunosuppressed patients, the incidence

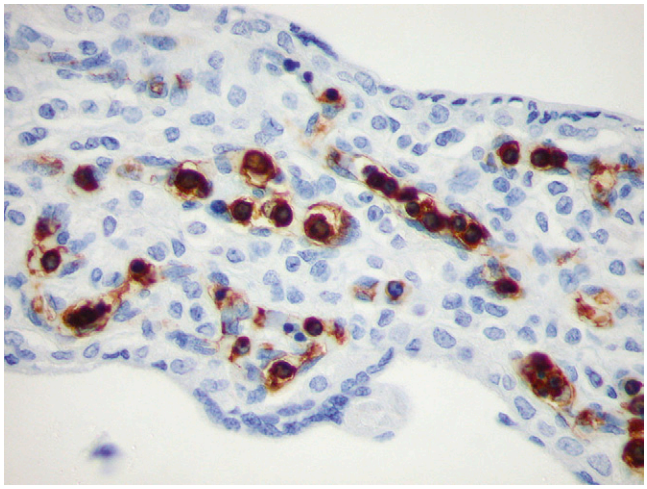

FIGURE 3.6 Hydrops fetalis caused by parvovirus B19 infection. Normoblasts within the villous capillaries show intranuclear viral antigen. (Immunoperoxidase staining with DAB and hematoxylin counterstain; ×400.)

of coinfection with other viruses is high, and the presence of adenovirus tends to be overlooked. Immunohistochemical staining has been of value in differentiating adenovirus colitis from CMV colitis.[70,75]

Parvovirus B19 Infection

Parvovirus B19 has been associated with asymptomatic infections, erythema infectiosum, acute arthropathy, aplastic crisis, hydrops fetalis, and chronic anemia and red cell aplasia. In addition, parvovirus B19 infection has been recognized as an important cause of severe anemia in immunocompromised leukemic patients receiving chemotherapy.[76]

The diagnosis of parvovirus infection can be achieved by identifying typical findings in bone marrow specimens, including decreased or absent red cell precursors, giant pronormoblasts, and eosinophilic or amphophilic intranuclear inclusions in erythroid cells.[77,78] Because intravenous immunoglobulin therapy is effective, a rapid and accurate diagnostic method is important. Immunohistochemistry with a monoclonal antibody against VP1 and VP2 capsid proteins has been used as a rapid and sensitive method to establish the diagnosis of parvovirus B19 infection in formalin-fixed, paraffin-embedded tissues.[79-82] Immunohistochemistry is of particular help in detecting parvovirus B19 antigen in cases with sparse inclusions, to study cases not initially identified by examination of routinely stained tissue sections, or in cases of hydrops fetalis with advanced cytolysis (Fig. 3.6).[79,83,84] Several studies have found a strong correlation among results obtained from morphologic, immunohistochemical, *in situ* hybridization (ISH), and polymerase chain reaction (PCR) methods.[78,79,82,84]

Viral Hemorrhagic Fevers

Since the 1980s, numerous emerging and reemerging agents of viral hemorrhagic fevers have attracted the attention of pathologists.[3-5] Investigators have played

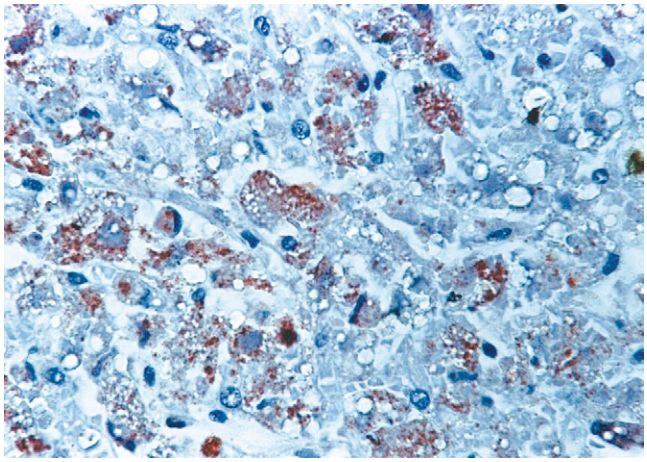

FIGURE 3.7 Yellow fever. Abundant yellow fever viral antigens are seen within hepatocytes and Kupffer cells. (Immunoperoxidase staining with AEC and hematoxylin counterstain; ×400.)

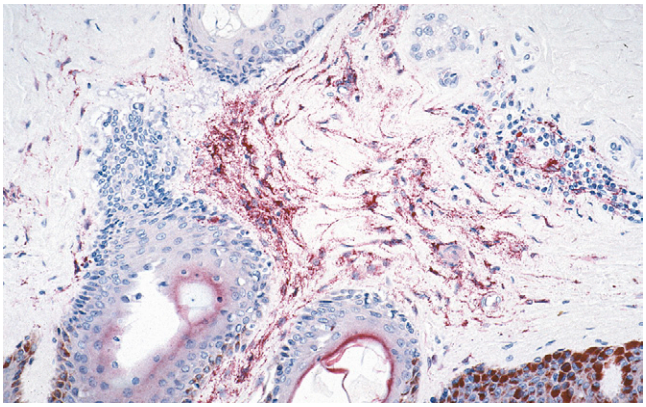

FIGURE 3.8 Extensive Ebola viral antigens are seen primarily within fibroblasts in the dermis of a skin specimen from a fatal case of Ebola hemorrhagic fever. (Immunoalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain; original magnification ×20.)

an important role in identifying these agents and in supporting epidemiologic, clinical, and pathogenetic studies of emerging viral hemorrhagic fevers.[4,5,7] Viral hemorrhagic fevers are often fatal. They are clinically difficult to diagnose (in the absence of bleeding or organ manifestations) and frequently require handling and testing of potentially dangerous biological specimens. In addition, histopathologic features are not pathognomonic, and they can resemble other viral, rickettsial, and bacterial (e.g., leptospirosis) infections. Immunohistochemistry is essential and has been successfully and safely applied to the diagnosis and study of the pathogenesis of these diseases.

Several studies have established the utility of IHC as a sensitive, safe, and rapid diagnostic method for the diagnosis of viral hemorrhagic fevers such as yellow fever (Fig. 3.7),[85-87] dengue hemorrhagic fever,[87,88] Crimean-Congo hemorrhagic fever,[89] Argentine hemorrhagic fever,[90] Venezuelan hemorrhagic fever,[91] and Marburg disease.[92] Additionally, a sensitive, specific, and safe immunostaining method has been developed to diagnose Ebola hemorrhagic fever in formalin-fixed skin biopsies (Fig. 3.8).[93] Immunohistochemistry demonstrated that Lassa virus targets primarily endothelial cells, mononuclear inflammatory cells, and hepatocytes (Fig. 3.9).[93-95]

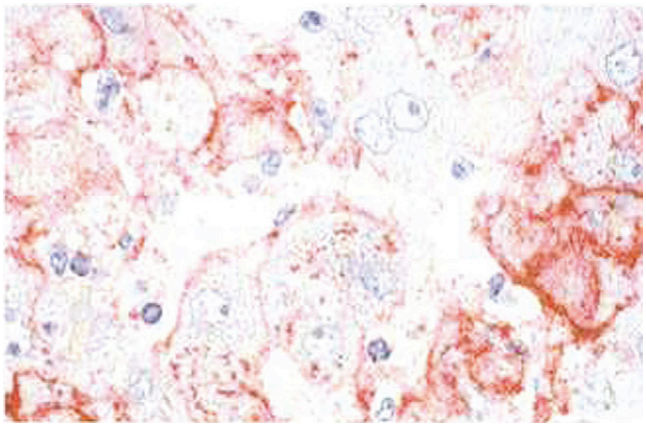

FIGURE 3.9 Lassa fever. Liver from a patient with Lassa fever. Scattered hepatocytes and reticuloendothelial cells show reactivity with monoclonal antibody to Lassa virus. (Naphthol fast red substrate and hematoxylin counterstain; original magnification ×100.)

Polyomaviruses

BK virus infections are frequent during infancy; in immunocompetent individuals the virus remains latent in the kidneys, central nervous system, and B lymphocytes. In immunocompromised patients, the infection reactivates and spreads to other organs. BK virus nephropathy is an important cause of graft failure in patients with a renal transplant,[96] with a prevalence varying from 2% to 4.5% in different transplant centers.[96,97] Since specific clinical signs and symptoms are lacking in BK virus nephropathy, the diagnosis can only be made histologically in a graft biopsy.[98] In the kidney, the infection is associated with mononuclear interstitial inflammatory infiltrates and tubular atrophy, findings that can be difficult to distinguish from acute rejection.[98] The cytopathic changes observed in BK virus infection are not pathognomonic and can be observed in other viral infections. Moreover, in early BK virus infection there are minimal or no histologic changes, although IHC can identify viral antigen.[99,100] In this setting, IHC with an antibody against the large T antigen of SV40 virus has been effective in demonstrating BK virus infection (Fig. 3.10).[96,99,101-103]

The human polyomavirus JC is a double-stranded DNA virus that causes progressive multifocal leukoencephalopathy (PML). This fatal demyelinating disease is characterized by cytopathic changes in oligodendrocytes and bizarre giant astrocytes. In addition to detection by antibodies to SV40-T antigen, IHC using a polyclonal rabbit antiserum against the protein VP1 is a specific, sensitive, and rapid method used to confirm the diagnosis of PML.[104-107] JC virus antigen is usually seen within oligodendrocytes (Fig. 3.11) and occasional astrocytes, and antigen-bearing cells are more commonly seen in early lesions.

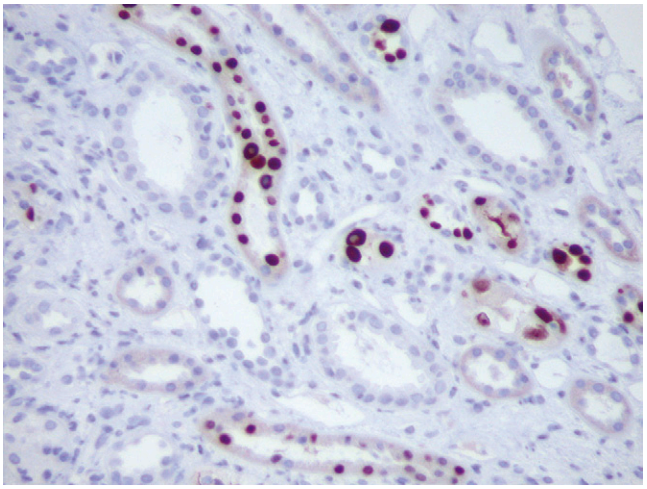

FIGURE 3.10 Immunohistochemical detection of SV40-T antigen in the nuclei of tubular cells in a renal transplant patient with BK virus–associated nephropathy. (Immunoperoxidase staining with DAB and hematoxylin counterstain; ×400.)

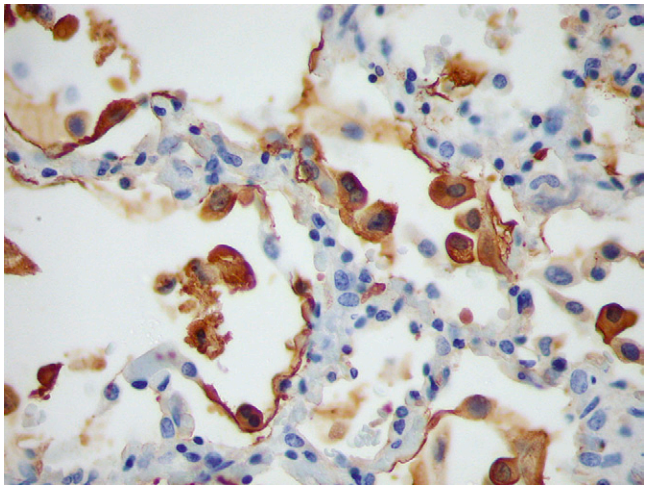

FIGURE 3.12 Immunostaining of RSV antigens in desquamated bronchial and alveolar lining cells using a monoclonal antibody. (Immunoperoxidase staining with DAB and hematoxylin counterstain; ×400.)

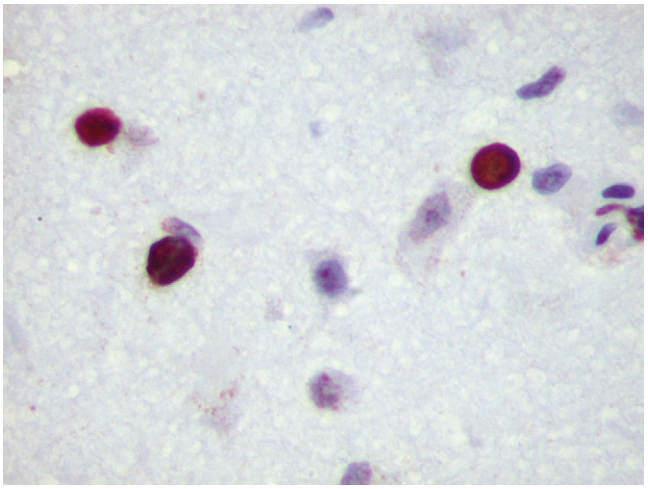

FIGURE 3.11 Progressive multifocal leukoencephalopathy: SV40-T antigen in the nuclei of enlarged oligodendrocytes in a patient with JC virus infection. (Immunoperoxidase staining with DAB and hematoxylin counterstain; ×400.)

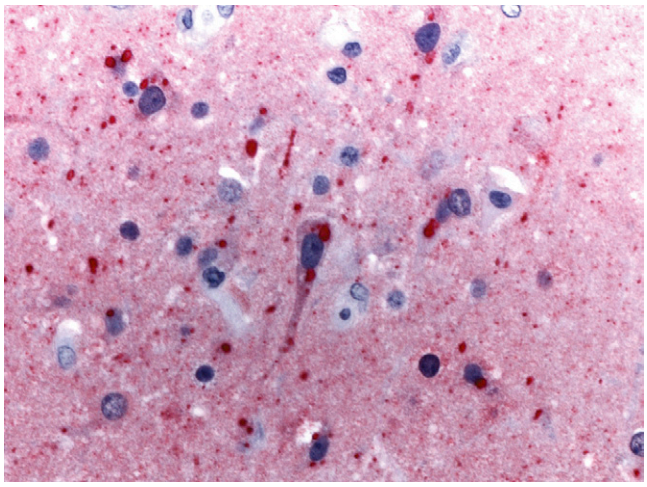

FIGURE 3.13 Immunostaining of rabies viral antigens in neurons of the CNS using a rabbit polyclonal antibody. Red precipitate corresponds to Negri inclusions by H&E staining. (Immunoalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain; original magnification ×40.)

Other Viral Infections

Immunohistochemistry has also been used to confirm the diagnosis of respiratory viral diseases such as influenza A virus and respiratory syncytial virus infections (Fig. 3.12) when cultures were not available.[108-111]

The diagnosis of rabies relies heavily on histopathologic examination of tissues to demonstrate its characteristic cytoplasmic inclusions (Negri bodies). In a significant percentage of cases, Negri bodies are inconspicuous and so few that confirming the diagnosis of rabies is extremely difficult.[112] Furthermore, in nonendemic areas the diagnosis of rabies usually is not suspected clinically, or the patient may present with ascending paralysis. In these settings, immunohistochemical staining is a very sensitive, specific, and safe diagnostic tool for rabies (Fig. 3.13).[112-116] Other viral agents that can be diagnosed using immunohistochemical methods include enteroviruses,[117-120] eastern equine encephalitis virus,[121-123] and rotavirus.[124-126]

Immunohistochemical staining has been used in the histopathologic diagnosis of viral hepatitis C; however, IHC for this virus is not as effective as serologic assays and detection of HCV RNA in serum.

BACTERIAL INFECTIONS

Among bacterial infections, the greatest number of immunohistochemical studies has been performed in the investigation of *Helicobacter pylori*. A few studies have evaluated the use of IHC for other bacterial, mycobacterial, rickettsial, and spirochetal infections.

Antigen retrieval is generally not required for the immunohistochemical demonstration of bacteria in

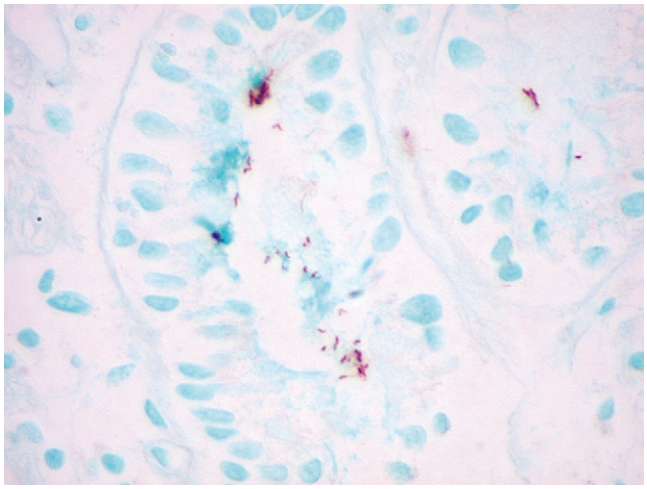

FIGURE 3.14 Numerous curved *H. pylori* in the superficial gastric mucus are clearly demonstrated by immunoperoxidase staining in this patient with chronic active gastritis. (Immunoperoxidase staining with DAB and hematoxylin counterstain; ×400.)

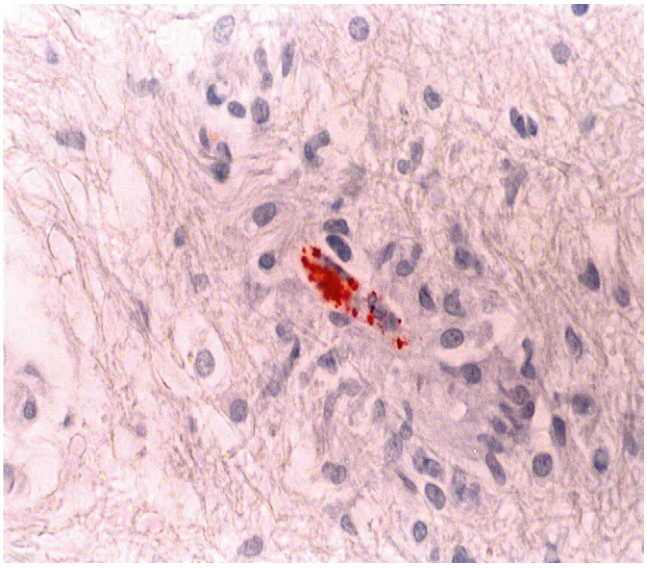

FIGURE 3.15 Immunohistologic demonstration of *R. rickettsii* within vascular endothelium in the pons of a patient with fatal Rocky Mountain spotted fever. (Immunoperoxidase staining with AEC and hematoxylin counterstain; ×600.)

fixed tissue. However, interpreting the results can be complicated because many of these antibodies cross-react with other bacteria. Moreover, antibodies may react with only portions of the bacteria, and they may label remnants of bacteria or spirochetes when viable organisms are no longer present.

Helicobacter Pylori Infection

Gastric infection by *H. pylori* results in chronic active gastritis and is strongly associated with lymphoid hyperplasia, gastric lymphomas, and gastric adenocarcinoma. Heavy infections with numerous organisms are easily detected on routine hematoxylin and eosin–stained tissues; however, the detection rate is only 66% with many false-positive and false-negative results.[127,128] Conventional histochemical methods such as silver stains are more sensitive than hematoxylin and eosin in detecting *H. pylori*. Nonetheless, for detecting scant numbers of organisms, it has been proven that IHC has high specificity and sensitivity, is less expensive when all factors are considered, is superior to conventional histochemical methods, and has a low interobserver variation (Fig. 3.14).[127] Treatment for chronic active gastritis and *H. pylori* infection can change the shape of the microorganism. This can make it difficult to identify and differentiate the organism from extracellular debris or mucin globules. In these cases IHC improves the rate of successful identification of the bacteria, even when histologic examination and cultures are falsely negative.[129-132]

Whipple's Disease

Whipple's disease affects primarily the small bowel and mesenteric lymph nodes and less commonly other organs such as the heart and central nervous system. Numerous foamy macrophages characterize the disease, and the diagnosis usually relies on the demonstration of PAS-positive intracytoplasmic bacteria. Nevertheless, the presence of PAS-positive macrophages is not pathognomonic; they can be observed in other diseases such as *Mycobacterium avium* infections, histoplasmosis, *Rhodococcus equi* infections, and macroglobulinemia. *Tropheryma whipplei* is a rare cause of endocarditis that shares many histologic features with other culture-negative endocarditides such as those caused by *Coxiella burnetii* and *Bartonella* sp.[133] The development of specific antibodies against these microorganisms has significantly enhanced the ability to detect them in the heart valves of patients with culture-negative endocarditis.[134] Immunohistochemical staining with rabbit polyclonal antibody provides a sensitive and specific method for the rapid diagnosis of intestinal and extraintestinal Whipple's disease and for follow-up of treatment response.[135-137]

Rocky Mountain Spotted Fever

Confirmation of Rocky Mountain spotted fever (RMSF) usually requires the use of serologic methods to detect antibodies to spotted fever group (SFG) Rickettsiae; however, most patients with RMSF lack diagnostic titers during the first week of disease. Immunohistochemistry has been successfully used to detect SFG Rickettsiae in formalin-fixed tissue sections, and it is superior to histochemical methods (Fig. 3.15).[138,139] Several studies illustrate the value of IHC in diagnosing suspected cases of RMSF using skin biopsies with high specificity and sensitivity, and in confirming fatal cases of seronegative RMSF.[10,140-144] *R. rickettsii* cannot be distinguished from other spotted fever group Rickettsiae such as *R. parkeri* or *R. conorii* because they cross-react.

Bartonella Infections

Bartonella are slow-growing, fastidious gram-negative, Warthin-Starry–stained bacteria associated with bacillary angiomatosis, peliosis hepatis, cat-scratch disease,

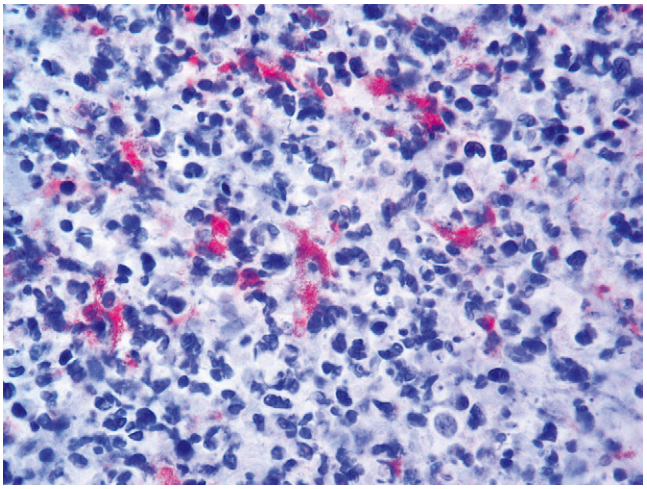

FIGURE 3.16 Photomicrograph of a lymph node biopsy from a patient with cat-scratch disease showing abundant extracellular, clumped coccobacilli of *B. henselae* in necrotic foci. (Immunoalkaline phosphatase with monoclonal antibody against *B. henselae,* naphthol fast red substrate and hematoxylin counterstain; ×200.) *Courtesy of Dr. Suimin Qiu, University of Texas Medical Branch.*

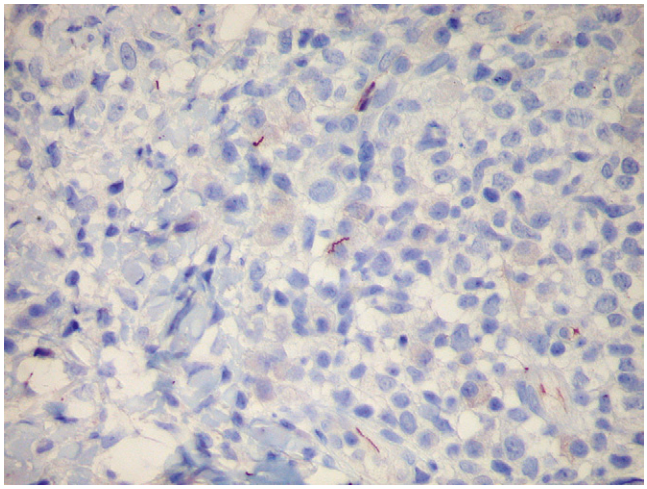

FIGURE 3.17 Syphilis. Skin biopsy from a patient with secondary syphilis. Scattered intact *T. pallidum* are easily visible with a rabbit polyclonal antibody against *T. pallidum.* (Immunoperoxidase staining with DAB and hematoxylin counterstain; ×400.)

trench fever, relapsing bacteremia, and disseminated granulomatous lesions of liver and spleen.[145] *Bartonella* are important agents of blood culture–negative endocarditis. Traditional techniques such as histology, electron microscopy, and serology have been employed to identify the agents of culture-negative endocarditis. However, *Bartonella* sp., *C. burnetii*, and *T. whipplei* endocarditis share many morphologic features that do not allow for a specific histologic diagnosis.[146] Besides, serologic tests for *Bartonella* sp. may show cross-reactivity with *C. burnetii* and *Chlamydia* sp.[147] Immunostaining has been successfully used to identify *Bartonella henselae* and *B. quintana* in the heart valves of patients with blood culture–negative endocarditis and has significantly enhanced the ability to establish a specific diagnosis in these cases.[148,149] A polyclonal rabbit antibody that does not differentiate between *B. henselae* and *B. quintana* has also been used to detect these microorganisms in cat-scratch disease (Fig. 3.16), bacillary angiomatosis, and peliosis hepatis.[150,151] A commercially available monoclonal antibody specific for *B. henselae* is also available and has been used to demonstrate the organism in a case of spontaneous splenic rupture caused by this bacterium.[152]

Syphilis

Syphilis continues to be a public health problem caused by *T. pallidum*, a fastidious organism that has not been cultivated.[153] The diagnosis of syphilis relies on serology and the identification of *T. pallidum* by dark-field microscopy. However, these methods have low sensitivity and specificity,[154] and serologic methods can be negative in early stages of the disease and in immunosuppressed patients such as those coinfected with human immunodeficiency virus (HIV).[155] In tissue sections, the usual method for detecting spirochetes is through silver impregnation stains (Warthin-Starry or Steiner). These stains, however, can be technically difficult to perform and interpret, are nonspecific, and frequently show marked background artifacts because silver stains also highlight melanin granules and reticulin fibers. Detection rates of spirochetes using silver stains vary from 33% to 71%.[156] It has been shown that immunostaining of biopsy specimens with anti–*T. pallidum* polyclonal antibody (Fig. 3.17) is more sensitive and specific than silver staining methods, with sensitivities ranging from 71% to 94%.[153,156,157]

Mycobacterium Tuberculosis Infection

Identification of *M. tuberculosis* is routinely achieved by acid-fast bacilli (AFB) staining, culture of biopsy specimens, or both. Nevertheless, AFB staining has a low sensitivity, and it is not specific because it does not differentiate mycobacterial species.[158] Furthermore, cultures may take several weeks, and sensitivity is low in paucibacillary lesions.[159] In the histologic diagnosis of mycobacterial infections, IHC with anti-BCG polyclonal antibody has shown better sensitivity than AFB staining. However, in paucibacillary lesions it is inferior to AFB staining and cannot differentiate between *M. tuberculosis* and other mycobacteria.[160] Recently a polyclonal antibody against the *M. tuberculosis*–secreted antigen MPT64 was used in cases of mycobacterial lymphadenitis. This method showed 90% sensitivity and 83% specificity and performed better than AFB staining in cases of paucibacillary disease and comparably to nested PCR.[161]

Other Bacterial Infections

Other bacterial diseases that can be identified by IHC in formalin-fixed tissue include leptospirosis, a zoonosis that usually presents as an acute febrile syndrome but occasionally can have unusual manifestations such as pulmonary hemorrhage with respiratory failure or abdominal pain.[162-164] Rabbit polyclonal antibodies

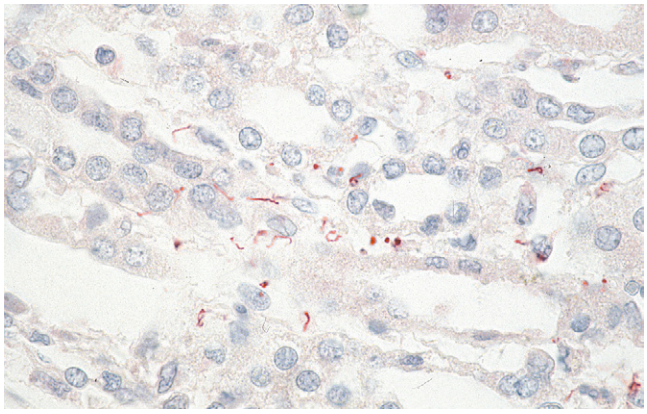

FIGURE 3.18 Leptospira. Immunostaining of intact leptospires and granular forms of leptospiral antigens in kidney of patient who died of pulmonary hemorrhage. (Immunoalkaline phosphatase with rabbit polyclonal antisera with naphthol fast red substrate and hematoxylin counterstain; original magnification ×63.)

have been used in IHC to detect leptospiral antigens in the gallbladder and lungs from patients with unusual presentations (Fig. 3.18).[162-165]

Lyme disease has protean clinical manifestations, and *Borrelia burgdoferi* is difficult to culture from tissues and fluids. In addition, cultures are rarely positive before 2 to 4 weeks of incubation. *Borrelia burgdorferi* can be identified in tissues by immunostaining with polyclonal or monoclonal antibodies. Although IHC is more specific than silver impregnation staining, the sensitivity of immunostaining is poor, and the microorganisms are difficult to detect owing to the low numbers present in tissue sections.[166,167]

Q fever is a zoonosis caused by *Coxiella burnetii* and is characterized by protean and non-specific manifestations. Acute Q fever can manifest as atypical pneumonia or granulomatous hepatitis, frequently with characteristic fibrin ring granulomas. This microorganism is recognized as one agent that causes blood culture–negative chronic endocarditis.[168] A monoclonal antibody has been used to specifically identify *C. burnetii* in cardiac valves of patients with chronic Q fever endocarditis.[12,169]

Recently IHC has been successfully used to identify *Streptococcus pneumoniae* in formalin-fixed organs with an overall sensitivity of 100% and a specificity of 71% when compared with cultures.[170] Immunohistochemical assays are used to identify *Clostridium* sp., *S. aureus,* and *S. pyogenes;*[171,172] *Haemophilus influenzae;*[173-175] *Chlamydia* species;[176-178] *Legionella pneumophila* and *L. dumoffii;*[179-181] *Listeria monocytogenes;*[182-184] *Salmonella;*[185,186] and rickettsial infections other than Rocky Mountain spotted fever such as boutonneuse fever, epidemic typhus, murine typhus,[187] rickettsialpox,[188,189] African tick bite fever,[138] and scrub typhus.[190]

FUNGAL INFECTIONS

The great majority of fungi are readily identified by hematoxylin and eosin staining alone or in combination with histochemical stains (e.g., periodic acid-Schiff [PAS], Gomori's methenamine silver [GMS]). However, these stains cannot distinguish morphologically similar fungi with potential differences in susceptibility to antimycotic drugs. In addition, several factors may influence the appearance of fungal elements, which may appear atypical in tissue sections because of steric orientation, age of the fungal lesion, effects of antifungal chemotherapy, type of infected tissue, and host immune response.[191] Currently the final identification of fungi relies on culture techniques; however, culture may take several days or longer to yield a definitive result, and surgical pathologists rarely have access to fresh tissue.

In past years, IHC has been used to identify various fungal elements in paraffin-embedded, formalin-fixed tissue.[192-194] Immunohistochemical methods have the advantage of providing rapid and specific identification of several fungi and allowing pathologists to identify unusual filamentous hyphal and yeast infections and to accurately distinguish them from confounding artifacts.[193,195] In addition, IHC allows pathologists to correlate microbiological and histologic findings of fungal infections and to distinguish them from harmless colonization. Immunohistochemistry can also be helpful when more than one fungus is present; in these cases dual immunostaining techniques can highlight the different fungal species present in the tissue.[196] An important limitation of IHC in the identification of fungi is the well-known, widespread occurrence of common antigens among pathogenic fungi that frequently results in cross-reactivity with polyclonal antibodies and even with some monoclonal antibodies.[193,195-197] Therefore, assessing cross-reactivity using a panel of fungi is a very important step in the evaluation of immunohistochemical methods.[193,194]

Candida species are often stained weakly with hematoxylin and eosin, and sometimes the yeast form may be difficult to differentiate from *Histoplasma capsulatum, Cryptococcus neoformans,* and even *Pneumocystis carinii.* Polyclonal and monoclonal antibodies against *Candida* genus antigens are sensitive and strongly reactive and do not show cross-reactivity with other fungi tested.[193,194,198,199] In particular, two monoclonal antibodies against *Candida albicans* mannoproteins show high sensitivity and specificity. Monoclonal antibody 3H8 recognizes primarily filamentous forms of *C. albicans,* whereas monoclonal antibody 1B12 highlights yeast forms.[199-203]

Identification of *Cryptococcus neoformans* usually is not a problem when the fungus produces a mucicarmine-positive capsule. However, infections by capsule-negative strains are more difficult to diagnose, and the disease can be confused with histoplasmosis, blastomycosis, or torulopsis. Also, in long-standing infections the yeast often appear atypical and fragmented. Polyclonal antibodies raised against *C. neoformans* yeast cells are sensitive and specific.[193,194] More recently, monoclonal antibodies have been produced that allow identification and differentiation of varieties of *C. neoformans* in formalin-fixed tissue. The antibodies are highly sensitive (97%) and specific (100%) and can differentiate *C. neoformans var. neoformans* from *C. neoformans var. gattii.*[204,205]

Sporothrix schenckii may be confused in tissue sections with *Blastomyces dermatitidis* and fungal agents of phaeohyphomycosis. In addition, yeast cells of *Sporothrix schenckii* may be sparsely present in tissues. Antibodies against yeast cells of *S. schenckii* are sensitive but demonstrate cross-reactivity with *Candida* species; however, after specific adsorption of the antibody with *Candida* yeast cells, the cross-reactivity of the antibodies is eliminated.[193,194]

Invasive aspergillosis is a frequent cause of fungal infection with high morbidity and mortality rates in immunocompromised patients.[206] The diagnosis is often difficult and relies heavily on histologic identification of invasive septate hyphae and culture confirmation. Nevertheless, several filamentous fungi such as *Fusarium* species, *Pseudallescheria boydii,* and *Scedosporium* species share similar morphology with *Aspergillus* species in hematoxylin and eosin–stained tissues.[207] A precise and rapid diagnosis of invasive aspergillosis is important because early diagnosis is associated with improved clinical response, and it allows planning of the correct duration and choice of antimycotic therapy. Researchers have shown that the yield of cultures in histologically proven cases is low, ranging from 25% to 50%.[206,208-211] Several polyclonal and monoclonal antibodies against *Aspergillus* antigens have been tested in formalin-fixed tissues with variable sensitivities, and most of them cross-react with other fungi.[197,212,213] More recently, monoclonal antibodies (WF-AF-1, 164G, and 611F) against *Aspergillus* galactomannan have shown high sensitivity and specificity in identifying *A. fumigatus, A. flavus,* and *A. niger* in formalin-fixed tissues without cross-reactivity with other filamentous fungi.[211,214,215]

Cysts and trophozoites of *Pneumocystis jirovecii* can be detected in bronchoalveolar lavage specimens using monoclonal antibodies that yield results that are slightly more sensitive than GMS, Giemsa, or Papanicolaou staining (Fig. 3.19).[194,216,217] Antibodies are most helpful in cases of extrapulmonary pneumocystosis or in the diagnosis of *P. jirovecii* pneumonia when atypical pathologic features are present (e.g., hyaline membranes or granulomatous pneumocystosis where microorganisms are usually very sparse).

Penicillium marneffei can cause a disseminated infection in immunocompromised patients.[218,219] Morphologically the organisms must be differentiated from *H. capsulatum, C. neoformans,* and *C. albicans*. The monoclonal antibody EBA-1 against the galactomannan of *Aspergillus* species cross-reacts with and detects *P. marneffei* in tissue sections.[209,220] Immunohistochemistry has also been used to detect *Blastomyces, Coccidioides,* and *Histoplasma*.[193,194,221] However, the antibodies have significant cross-reactivity with several other fungi.

PROTOZOAL INFECTIONS

Protozoa usually can be identified in tissue sections stained with hematoxylin and eosin or Giemsa stain; however, because of the small size of the organisms and the subtle distinguishing features, an unequivocal diagnosis cannot always be made. The role of IHC in the detection of protozoal infections has been particularly valuable in cases in which the morphology of the parasite is distorted by tissue necrosis or autolysis. In addition, in immunocompromised patients, toxoplasmosis can have an unusual disseminated presentation with numerous tachyzoites without bradyzoites (Fig. 3.20).[222,223] Immunohistochemistry has also been useful in cases with unusual presentation of the disease.[224]

The diagnosis of leishmaniasis in routine practice usually is not difficult; however, in certain circumstances pathologic diagnosis may be problematic, as is the case in chronic granulomatous leishmaniasis with small numbers of parasites, when the microorganism presents in unusual locations, or when necrosis distorts the morphologic appearance of the disease.[225] In these cases, immunohistochemical staining has been a valuable diagnostic tool.[225-228] The highly sensitive and specific monoclonal antibody p19-11 recognizes different

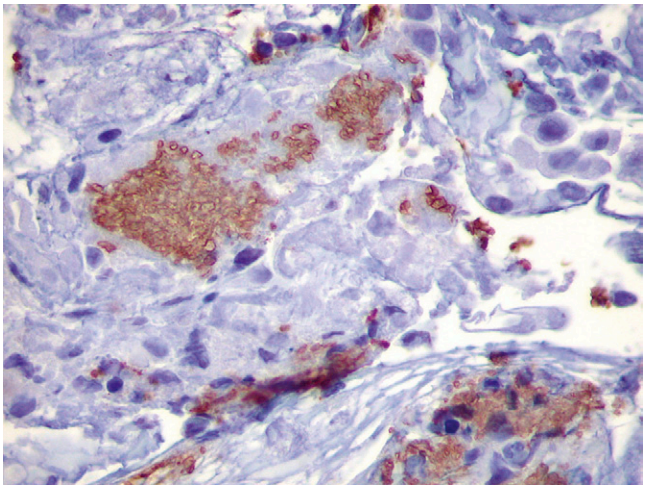

FIGURE 3.19 Immunodeficient patient with *P. jirovecii* pneumonia. Cohesive aggregates of cyst forms and trophozoites within alveolar spaces are demonstrated by a monoclonal antibody against pneumocystis with an immunoperoxidase technique. (Immunoperoxidase staining with DAB and hematoxylin counterstain; ×400.)

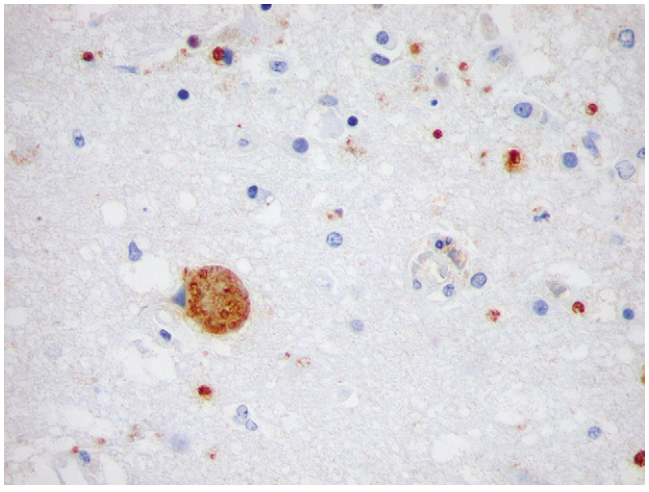

FIGURE 3.20 HIV-infected patient with toxoplasmic encephalitis. Immunoperoxidase staining highlights cyst forms and scattered tachyzoites. (DAB substrate with hematoxylin counterstain; ×400.)

species of *Leishmania* and allows differentiation from morphologically similar microorganisms (*Toxoplasma, Trypanosoma cruzi,* and *P. marneffei*).[225]

Immunohistochemical assays using polyclonal antibodies specific for *Balamuthia mandrillaris, Naegleria fowleri,* and *Acanthamoeba* sp. are used to demonstrate amebic trophozoites and cysts in areas of necrosis and can allow their differentiation from macrophages in cases of amebic meningoencephalitis.[229]

Immunohistochemistry has also been used to identify *Cryptosporidium,*[230] *Entamoeba histolytica,*[231] *Trypanosoma cruzi,*[232-234] babesia,[235] *Giardia lamblia,*[236] *Plasmodium falciparum,* and *P. vivax* in fatal cases of malaria[237] in formalin-fixed paraffin-embedded tissue samples.

EMERGING INFECTIOUS DISEASES

In 1992 the Institute of Medicine defined emerging infectious diseases (EIDs) as caused by new, previously unidentified microorganisms or those whose incidence in humans has increased within the previous two decades or threatens to increase in the near future.[238] The list of pathogens newly recognized since 1973 is long and continues to increase. Recognizing emerging infections is a challenge, and many new infectious agents remain undetected for years before emerging as an identified public health problem.[239] EIDs are a global phenomenon that requires a global response. The Centers for Disease Control and Prevention (CDC) has defined the strategy to prevent and detect EIDs.[239] The anatomic pathology laboratory plays a critical role in the initial and rapid detection of EIDs.[240,241] Besides assisting in the identification of new infectious agents, IHC has contributed to the understanding of the pathogenesis and epidemiology of EIDs.

Hantavirus Pulmonary Syndrome

In 1993 in the southwestern United States, several previously healthy individuals died of rapidly progressive pulmonary edema, respiratory insufficiency, and shock.[242,243] Immunohistochemistry played a central role in identifying the viral antigens of an unknown hantavirus,[244,245] detecting the occurrence of unrecognized cases of hantavirus pulmonary syndrome prior to 1993, and showing the distribution of viral antigen in endothelial cells of the microcirculation, particularly in the lung (Fig. 3.21).[244,246]

West Nile Virus Encephalitis

West Nile virus (WNV) was originally identified in Africa in 1937, and the first cases of WNV encephalitis in the United States were described in 1999. The clinical picture is variable and non-specific. It can range from subclinical infection to flaccid paralysis and encephalitis characterized morphologically by perivascular mononuclear cell inflammatory infiltrates, neuronal necrosis, edema, and microglial nodules, particularly prominent in the brainstem, cerebellum, and spinal cord.[247-251] The diagnosis of WNV encephalitis is usually established by identifying virus-specific IgM in CSF and/or serum and by demonstrating viral RNA in serum, CSF, or other tissue.[252] Immunostaining with monoclonal or polyclonal antibodies has been successfully used to diagnose WNV infection in immunocompromised patients with an inadequate antibody response (Fig. 3.22).[248]

Enterovirus 71 Encephalomyelitis

Enterovirus 71 (EV71) has been associated with hand, foot, and mouth disease; herpangina; aseptic meningitis; and poliomyelitis-like flaccid paralysis. More recently EV71 has been associated with unusual cases of fulminant encephalitis, pulmonary edema and hemorrhage, and heart failure.[253,254] Severe and extensive encephalomyelitis of the cerebral cortex, brainstem, and spinal cord has been described. Immunohistochemical staining with monoclonal antibody against EV71 has played a pivotal role in the linking of EV71 infection to fulminant encephalitis (Fig. 3.23). Viral antigen is observed within

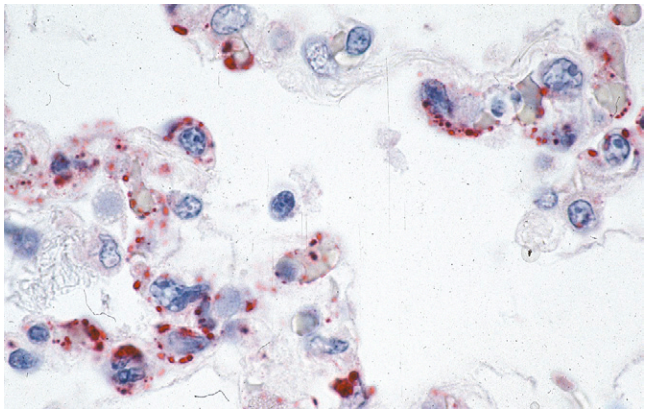

FIGURE 3.21 Hantavirus antigen–containing endothelial cells of the pulmonary microvasculature in the lung of an HPS patient as detected by immunohistochemistry using a mouse monoclonal antibody. (Immunoalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain; original magnification ×100.)

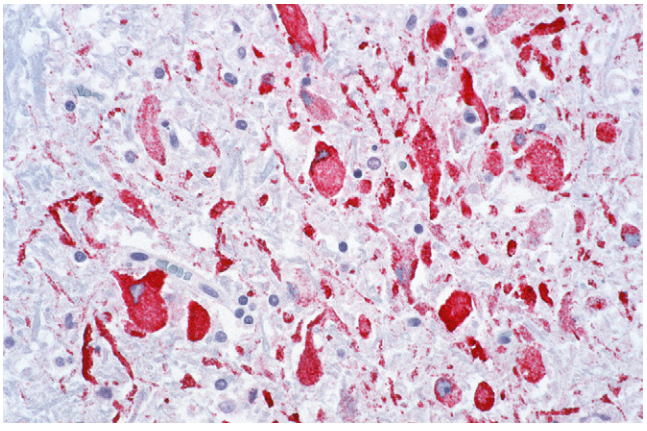

FIGURE 3.22 West Nile virus. Immunostaining of flaviviral antigens in neurons and neuronal processes in the central nervous system from an immunosuppressed patient who died of West Nile virus encephalitis. (Flavivirus-hyperimmune mouse ascitic fluid naphthol fast red substrate with hematoxylin counterstain; original magnification ×40.)

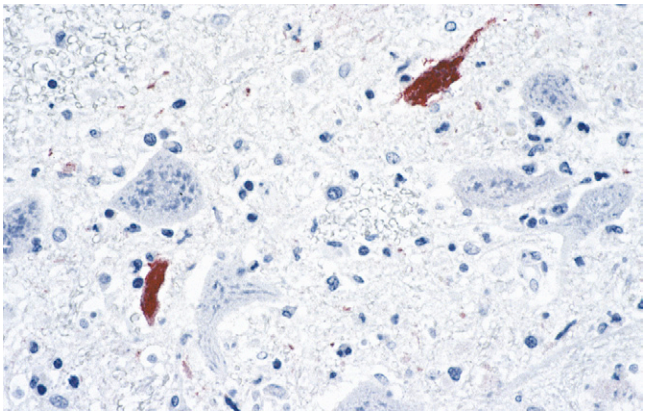

FIGURE 3.23 Enterovirus 71. Positive staining of EV71 viral antigens in neurons and neuronal processes of a fatal case of enterovirus encephalitis. (Immunoalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain; original magnification ×40.)

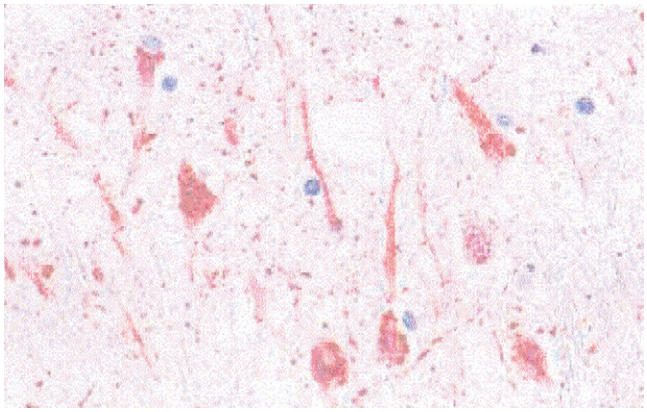

FIGURE 3.24 Nipah virus. Immunostaining of Nipah virus antigens in neurons and neuronal processes in CNS of a fatal case of Nipah virus encephalitis. (Immunoalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain; original magnification ×63.)

neurons, neuronal processes, and mononuclear inflammatory cells.[255-257]

Nipah Virus Infection

Nipah virus is a recently described paramyxovirus that causes an acute febrile encephalitic syndrome with a high mortality rate.[258-260] Pathology played a key role in identifying the causative agent. Histopathologic findings include vasculitis with thrombosis, microinfarctions, syncytial giant cells, and viral inclusions.[258,260] Although characteristic of this disease, syncytial giant endothelial cells are seen only in 25% of cases,[258] and viral inclusions of similar morphology can been seen in other paramyxoviral infections. Immunostaining provides a useful tool for unequivocal diagnosis of the disease, demonstrating viral antigen within neurons and endothelial cells of most organs (Fig. 3.24).[5,258]

Ehrlichioses

Tick-transmitted intracellular gram-negative bacteria belonging to the genera *Ehrlichia* and *Anaplasma* are the agents of human monocytotropic ehrlichiosis and human granulocytotropic anaplasmosis, respectively. The acute febrile illnesses usually present with cytopenias, myalgias, and mild to moderate hepatitis.[261-264]

Diagnosis of ehrlichiosis depends upon finding the characteristic monocytic and/or granulocytic cytoplasmic inclusions (morulae), PCR analysis of blood, and detection of specific antibodies in blood. However, morulae are rare and often missed on initial evaluation; hematoxylin and eosin–stained sections often fail to show organisms even when IHC reveals abundant ehrlichial antigen; and antibody titers may take several weeks to rise to diagnostic levels.[261,265] Additionally, immunocompromised patients may not develop antiehrlichial antibodies prior to death.[261,263] In these cases, immunostaining for *Ehrlichia* or *Anaplasma* is a sensitive and specific diagnostic method.[261,263,264,266]

Immunohistochemistry has been a very valuable tool used to identify and study several other EIDs such as Ebola hemorrhagic fever;[93-95] Hendra virus encephalitis;[5,267,268] leptospirosis;[163-165] emerging tick-borne rickettsioses such as *R. parkeri*[269] and *R. africae*;[270] and, recently, a new coronavirus associated with severe acute respiratory syndrome (SARS).[271,272] SARS was first recognized during a global outbreak of severe pneumonia that occurred in late 2002 in Guangdong Province, China, and then erupted in February 2003 with cases in more than two dozen countries in Asia, Europe, North America, and South America. Early in the investigation, clinical, pathologic, and laboratory studies focused on previously known agents of respiratory illness. Subsequently, a virus was isolated from the oropharynx of a SARS patient and identified by ultrastructural characteristics as belonging to the family Coronaviridae.[271,271] Various reports have described diffuse alveolar damage as the main histopathologic finding in SARS patients, and SARS-associated coronavirus (SARS-CoV) has been demonstrated in human and experimental animal tissues by immunohistochemical (Fig. 3.25) or ISH assays.[273-282]

PATHOLOGISTS, IMMUNOHISTOCHEMISTRY, AND BIOTERRORISM

There is increasing concern about the use of infectious agents as potential biological weapons. Biological warfare agents vary from rare exotic viruses to common bacterial agents, and the intentional use of biologic agents to cause disease can simulate naturally occurring outbreaks or may have unusual characteristics.[283] The CDC has issued recommendations for a complete public health response to a biological attack.[284-286] Two important components of this response plan include the rapid diagnosis and characterization of biological agents. Pathologists using newer diagnostic techniques such as IHC, ISH, and PCR will have a direct impact on

the rapid detection and control of emerging infectious diseases from natural or intentional causes.[287] Immunohistochemistry provides a simple, safe, sensitive, and specific method for the rapid detection of biological threats (at the time of investigation or retrospectively), thereby facilitating the rapid implementation of effective public health responses.

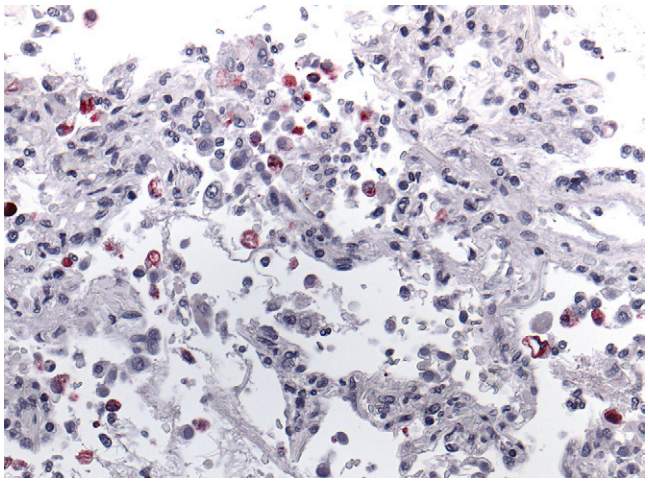

FIGURE 3.25 SARS. Coronavirus antigen–positive pneumocytes and macrophages in the lung of a SARS case. (Immunoalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain; original magnification ×63.)

Anthrax

Immunohistochemical staining of *Bacillus anthracis* with monoclonal antibodies against cell wall and capsule antigens has been successfully used in the recognition of bioterrorism-related anthrax cases and is an important step in early diagnosis and treatment (Fig. 3.26A-C).[5,288-292] Gram staining and culture isolation of *B. anthracis* are usually used to diagnose anthrax; however, previous antibiotic treatment will affect culture yield and Gram stain identification of the bacteria.[290] Immunohistochemistry has demonstrated high sensitivity and specificity for the detection of *B. anthracis* in skin biopsies, pleural biopsies, transbronchial biopsies, and pleural fluids (see Fig. 3.26).[289-291,293]

In addition, immunostaining has been very useful for determining the route of entry of the bacteria and identifying the mode of spread of the disease.[290,294]

Tularemia

Immunohistochemical staining is also valuable for rapid identification of *Francisella tularensis* in formalin-fixed tissue sections. Tularemia can have a variable clinical and pathologic presentation that can simulate other infectious diseases such as anthrax, plague, cat-scratch disease, or lymphogranuloma venereum. Moreover, the microorganisms are difficult to demonstrate in tissue sections, even with Gram stain or silver staining methods. A mouse monoclonal antibody against the

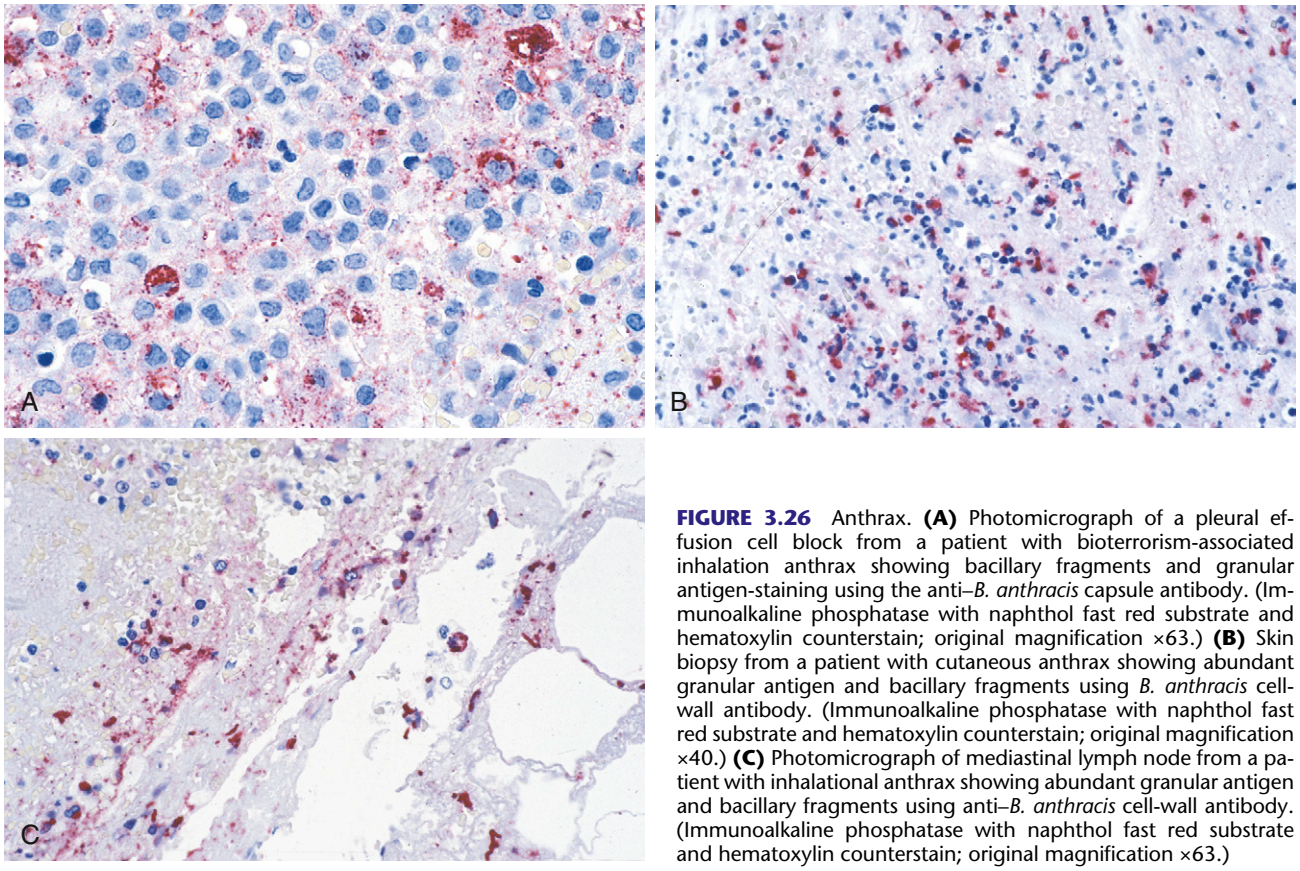

FIGURE 3.26 Anthrax. **(A)** Photomicrograph of a pleural effusion cell block from a patient with bioterrorism-associated inhalation anthrax showing bacillary fragments and granular antigen-staining using the anti–*B. anthracis* capsule antibody. (Immunoalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain; original magnification ×63.) **(B)** Skin biopsy from a patient with cutaneous anthrax showing abundant granular antigen and bacillary fragments using *B. anthracis* cell-wall antibody. (Immunoalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain; original magnification ×40.) **(C)** Photomicrograph of mediastinal lymph node from a patient with inhalational anthrax showing abundant granular antigen and bacillary fragments using anti–*B. anthracis* cell-wall antibody. (Immunoalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain; original magnification ×63.)

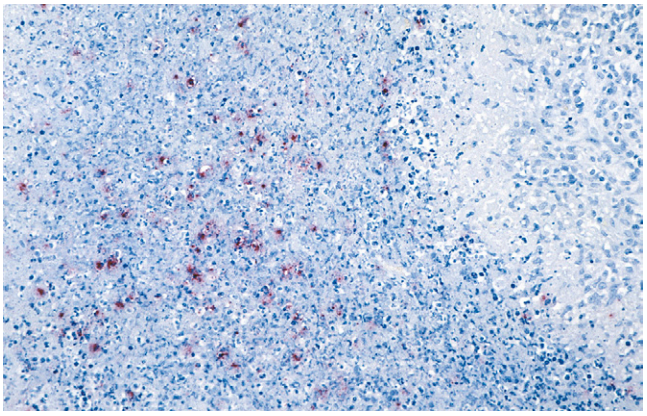

FIGURE 3.27 Tularemia. Immunohistochemistry of lymph node showing a stellate abscess with *F. tularensis* antigen-bearing macrophages in the central necrotic area using a mouse monoclonal antibody against the lipopolysaccharide of *F. tularensis*. (Immunoalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain; original magnification ×40.)

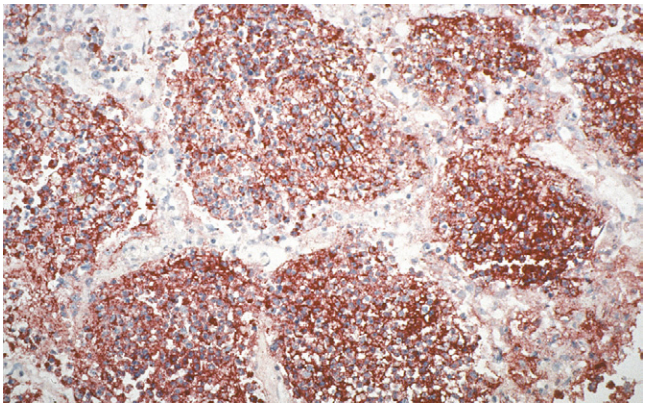

FIGURE 3.28 Immunohistochemical stain of lung containing abundant bacterial and granular *Yersinia pestis* antigen in the alveolar spaces using a mouse monoclonal antibody against F1 capsular antigen. (Immunoalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain; original magnification ×20.)

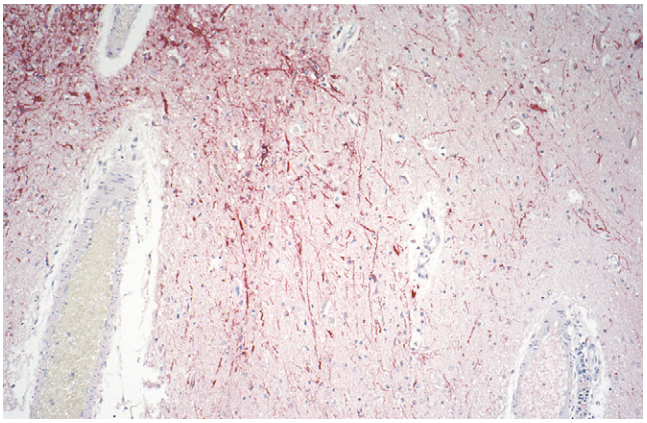

FIGURE 3.29 Immunostaining of viral antigens in neurons and neuronal processes in CNS using a mouse anti-eastern equine encephalitis virus antibody. (Immunoalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain; original magnification ×10.)

lipopolysaccharide of *F. tularensis* has been used with high sensitivity and specificity to demonstrate intact bacteria and granular bacterial antigen in the lungs, spleen, lymph nodes, and liver (Fig. 3.27).[295,296]

Plague

A mouse monoclonal antibody directed against the fraction 1 antigen of *Yersinia pestis* has been used to detect intracellular and extracellular bacteria in dermal blood vessels, lungs, lymph nodes, spleen, and liver (Fig. 3.28).[297-302] This technique is potentially useful for the rapid diagnosis of plague in formalin-fixed skin biopsies. In addition, IHC can differentiate primary and secondary pneumonic plague by identifying *Y. pestis* in different lung locations (e.g., alveoli vs. interstitium).[297]

Immunohistochemical methods using polyclonal or monoclonal antibodies have been used to identify several other potential biological terrorism agents, including the causative agents of brucellosis,[5] Q fever,[5,138,168,169] viral encephalitides (eastern equine encephalitis) (Fig. 3.29),[5,121-123] rickettsioses (typhus and Rocky Mountain spotted fever),[138-141,187] and viral hemorrhagic fevers (Ebola and Marburg hemorrhagic fever).[5,89-95]

BEYOND IMMUNOHISTOLOGY: MOLECULAR DIAGNOSTIC APPLICATIONS

Since the 1980s, an enormous advancement in molecular technology has dramatically influenced the diagnosis and study of infectious diseases. The application of molecular probes to the study and diagnosis of infectious diseases is a great adjunct to IHC as a diagnostic method and often allows for even more rapid and specific identification of organisms.[303-309] Along with rapid advances in molecular diagnostic techniques, there has been an increased interest in the use of paraffin-embedded specimens for nucleic acid hybridization assays. The two main hybridization formats used in the diagnostic pathology laboratory for the diagnosis of infectious diseases are ISH and PCR. *In situ* hybridization is analogous to IHC in that it allows the cellular identification and localization of microbial pathogens. Instead of microbial antigens, the targets of ISH are specific RNA or DNA sequences. Many viruses (Figs. 3.30-3.33), bacteria (Fig. 3.34), and other microorganisms can be localized in tissues by ISH; these include Epstein-Barr virus, HPV (Fig. 3.35), polyomaviruses (Fig. 3.36), *Mycobacterium leprae, Legionella, Haemophilus influenzae*, zygomycetes, and *Aspergillus*.[108,114,207,261,282,310-332] PCR has the advantage of increased sensitivity, minimal tissue requirements, and potential sequencing of the amplified product for specific identification of the microbial genotype or strain of the agent involved. There are PCR assays for most microorganisms that have been or can be adapted for use on formalin-fixed tissues.[11,124,172,293,333-345]

In summary, IHC, ISH, and PCR should be regarded as complementary diagnostic methods for use in the diagnostic pathology laboratory. The laboratory must

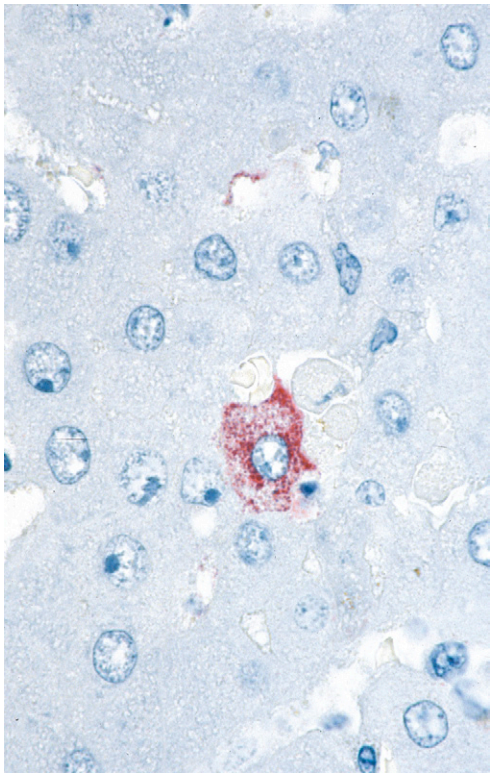

FIGURE 3.30 Crimean-Congo hemorrhagic fever (CCHF). Localization of CCHF viral RNA as seen in a single CCHF-infected hepatocyte. (Immunoalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain; original magnification ×250.)

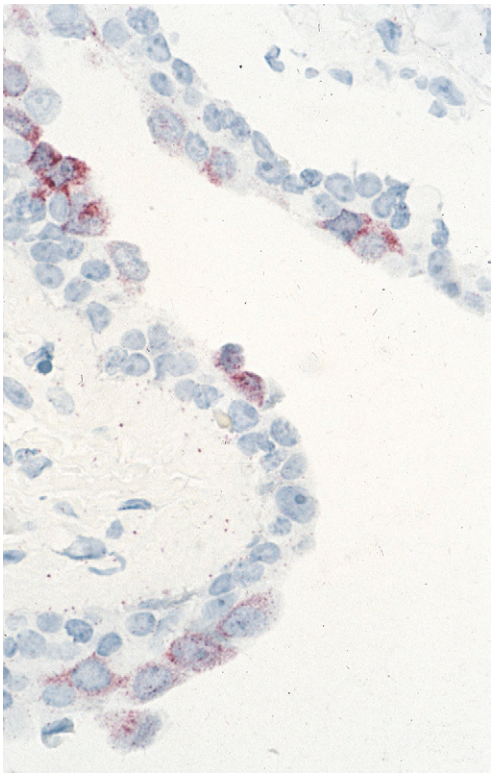

FIGURE 3.31 Influenza A. *In situ* hybridization showing localization of viral nucleic acids in bronchial epithelium using an influenza A hemagglutinin digoxigenin-labeled probe. (Immunoalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain; original magnification ×158.)

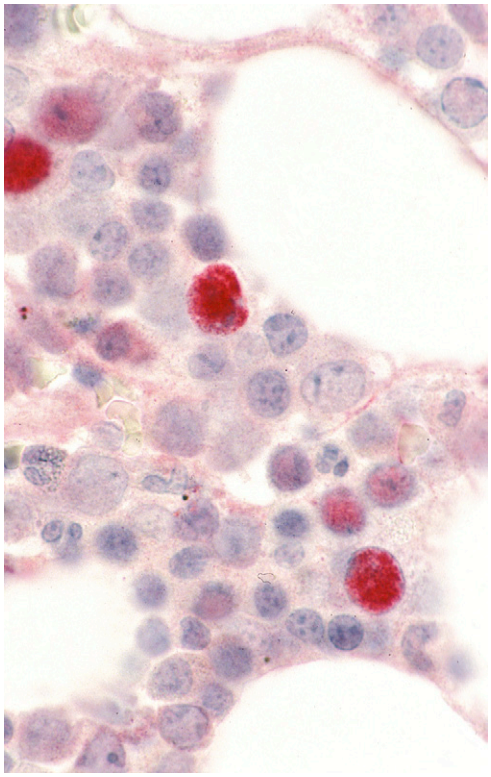

FIGURE 3.32 Parvovirus infection. Confirmation of B19-infected cells in bone marrow of an HIV-infected patient by using a digoxigenin-labeled B19 riboprobe and *in situ* hybridization. Staining is mainly nuclear and seen in multiple cells containing classic parvovirus inclusions. (Immunoalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain; original magnification ×250.)

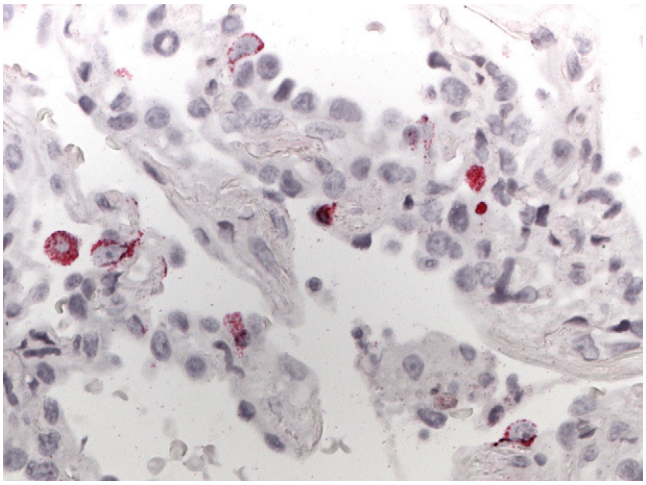

FIGURE 3.33 SARS. Lung showing diffuse alveolar damage and SARS-CoV nucleic acids primarily in pneumocytes as seen by colorimetric ISH. (Immunoalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain; original magnification ×158.)

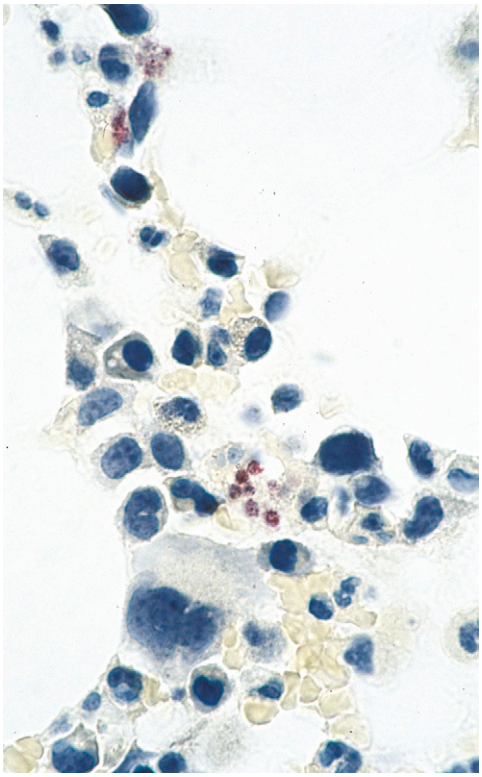

FIGURE 3.34 *Ehrlichia chaffeensis*. Organisms appear as red inclusions within monocytes by *in situ* hybridization. (Immunoalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain; original magnification ×250.)

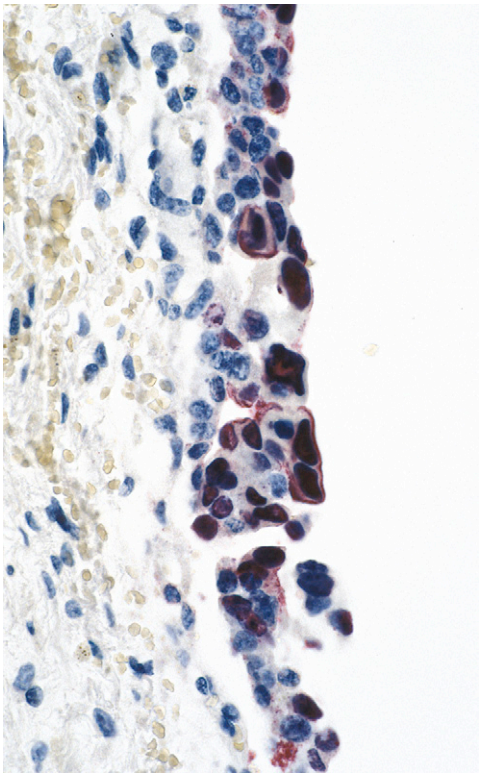

FIGURE 3.36 Polyomavirus (BK virus) infection. BK virus–infected cells in urothelium as seen by using a colorimetric ISH and a DNA probe. Staining is both nuclear and cytoplasmic and seen in multiple cells containing classic viral inclusions. (Immunoalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain; original magnification ×100.)

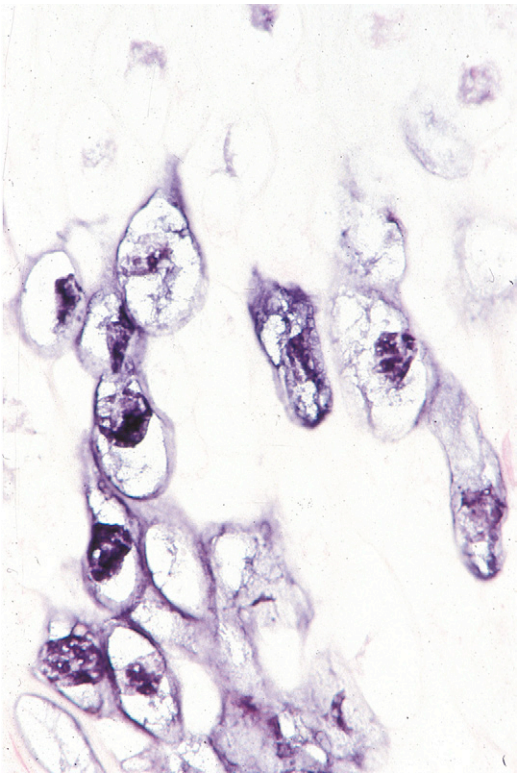

FIGURE 3.35 Human papillomavirus (HPV). *In situ* hybridization for HPV in a patient with a benign cervical lesion. HPV RNA is localized within nucleus and cytoplasm of koilocytotic cells. (Immunoalkaline phosphatase with naphthol fast red substrate and hematoxylin counterstain; original magnification ×250.)

consider the advantages and limitations of each method and how they apply to each case and the common needs of the laboratory. This ever-expanding field behooves all pathologists interested in the field of infectious diseases to keep abreast of the changing technology and its ever-increasing application in the arena of diagnosis.

REFERENCES

1. Cartun RW. Use of immunohistochemistry in the surgical pathology laboratory for the diagnosis of infectious diseases. *Pathol Case Rev*. 1999;4:260-265.
2. Watts JC. Surgical pathology in the diagnosis of infectious diseases (Editorial). *Am J Clin Pathol*. 1994;102:711-712.
3. Schwartz DA, Bryan RT. Infectious disease pathology and emerging infections: Are we prepared? *Arch Pathol Lab Med*. 1996;120:117-124.
4. Schwartz DA. Emerging and reemerging infections: Progress and challenges in the subspecialty of infectious disease pathology. *Arch Pathol Lab Med*. 1997;121:776-784.
5. Zaki SR, Paddock CD. The emerging role of pathology in infectious diseases. In: Scheld WM, Armonstrong D, Hughes JM, eds. *Emerging Infections 3*. Washington, D.C.: ASM Press; 1999:181-200.
6. Medical Examiners, Coroners, and Biologic Terrorism: A Guidebook for Surveillance and Case Management. *MMWR Morbidity and Mortality Weekly Report*. 2004;53(RR-8):1-53.
7. Zaki SR, Peters CJ. Viral hemorrhagic fevers. In: Connor DH, Chandler FW, Schwartz DA, Manz HJ, Lack EE, eds. *Pathology of Infectious Diseases*. Stamford, CT: Appleton & Lange; 1997:347-364.

8. Coons AH, Creech HJ, Jone RN, et al. The demonstration of pneumococcal antigen in tissues by use of fluorescent antibodies. *J Immunol*. 1942;45:159-170.
9. Cohen PR. Tests for detecting herpes simplex virus and varicella-zoster virus infections. *Dermatol Clin*. 1994;12:51-68.
10. Procop GW, Burchette Jr JL, Howell DN, et al. Immunoperoxidase and immunofluorescent staining of Rickettsia rickettsii in skin biopsies. *Arch Pathol Lab Med*. 1997;121:894-899.
11. Guarner J, Greer PW, Whitney A, et al. Pathogenesis and diagnosis of human meningococcal disease using immunohistochemical and PCR assay. *Am J Clin Pathol*. 2004;122:754-764.
12. Lepidi H, Houpikina P, Liang Z, et al. Cardiac valves in patients with Q fever endocarditis: microbiological, molecular, and histologic studies. *J Infect Dis*. 2003;187:1097-1106.
13. Eyzaguirre EJ, Haque AK. Application of immunohistochemistry to infections. *Arch Pathol Lab Med*. 2008;132:424-431.
14. Lepidi H, Fenollar F, Dumler JS. Cardiac valves in patients with Whipple endocarditis: microbiological, molecular, quantitative histologic, and immunohistochemical studies of 5 patients. *J Infect Dis*. 2004;190:935-945.
15. Jeavons L, Hunt L, Hamilton A. Immunochemical studies of heat-shock protein 80 of Histoplasma capsulatum. *J Med Vet Mycol*. 1994;32:47-57.
16. Werner M, Chott A, Fabiano A, et al. Effect of formalin tissue fixation and processing on immunohistochemistry. *Am J Surg Pathol*. 2000;24:1016-1019.
17. Woods GL, Walker DH. Detection of infection or infectious agents by use of cytologic and histologic stains. *Clin Microbiol Rev*. 1996;9:382-404.
18. Chandler FW. Invasive microorganisms. In: Spicer SS, ed. *Histochemistry in Pathology Diagnosis*. New York, NY: Marcel Dekker; 1987:77-101.
19. Clausen PP, Thomsen P. Demonstration of hepatitis B surface antigen in liver biopsies. A comparative investigation of immunoperoxidase and orcein staining on identical sections on formalin-fixed, paraffin-embedded tissue. *Acta Pathol Microbiol Scand [A]*. 1978;86A:383.
20. Thomsen P, Clausen PP. Occurrence of hepatitis B-surface antigen in a consecutive material of 1539 liver biopsies. *Acta Pathol Microbiol Immunol Scand [A]*. 1983;91:71.
21. Al Adnani MS, Ali SM. Patterns of chronic liver disease in Kuwait with special reference to localization of hepatitis B surface antigen. *J Clin Pathol*. 1984;37:549.
22. Taylor C. Lung, pancreas, colon and rectum, stomach, liver. In: Taylor CR, Cote RJ, eds. *Immunomicroscopy: A Diagnostic Tool for the Surgical Pathologist*. 2nd ed. Philadelphia: Saunders; 1994:292-317.
23. Park YN, Han KH, Kim KS, et al. Cytoplasmic expression of hepatitis B core antigen in chronic hepatitis B virus infection: role of precore stop mutants. *Liver*. 1999;19:199-205.
24. McDonald JA, Harris S, Waters JA, et al. Effect of human immunodeficiency virus (HIV) infection on chronic hepatitis B hepatic viral display. *J Hepatol*. 1987;4:337-342.
25. Hubscher SG, Portmann BC. Transplantation pathology. In: Burt AD, Portmann BC, Ferrell LD, eds. *MacSween's Pathology of the Liver*. 5th ed. Philadelphia: Elsevier; 2007:815-879.
26. Feiden W, Borchard F, Burrig KF, et al. Herpes esophagitis: I. Light microscopical immunohistochemical investigations. *Virchows Arch [A]*. 1984;404:167-176.
27. Nikkels AF, Delvenne P, Sadzot-Delvaux C, et al. Distribution of varicella zoster virus and herpes simplex virus in disseminated fatal infections. *J Clin Pathol*. 1996;49:243-248.
28. Greenson JK, Beschorner WE, Boitnott JK, et al. Prominent mononuclear cell infiltrate is characteristic of herpes esophagitis. *Hum Pathol*. 1991;22:541-549.
29. Wang JY, Montone KT. A rapid simple in situ hybridization method for herpes simplex virus employing a synthetic biotin-labeled oligonucleotide probe: a comparison with immunohistochemical methods for HSV detection. *J Clin Lab Anal*. 1994;8:105-115.
30. Kobayashi TK, Ueda M, Nishino T, et al. Brush cytology of herpes simplex virus infection in oral mucosa: use of the ThinPrep processor. *Diag Cytopath*. 1998;18:71-75.
31. Nicoll JAR, Love S, Burton PA, et al. Autopsy findings in two cases of neonatal herpes simplex virus infection: detection of virus by immunohistochemistry, in situ hybridization and the polymerase chain reaction. *Histopathology*. 1994;24:257-264.
32. Nikkels AF, Debrus S, Sadzot-Delvaux C, et al. Comparative immunohistochemical study of herpes simplex and varicella-zoster infections. *Virchows Archiv A Pathol Anat*. 1993;422:121-126.
33. Cohen PR. Tests for detecting herpes simplex virus and varicella-zoster virus infections. *Dermat Clin*. 1994;12:51-68.
34. Katano H, Sato Y, Kurata T, et al. Expression and localization of human herpesvirus 8-encoded proteins in primary effusion lymphoma, Kaposi's sarcoma, and multicentric Castleman's disease. *Virology*. 2000;269:335-344.
35. Katano H, Suda T, Morishita Y, et al. Human herpesvirus 8-associated solid lymphomas that occur in AIDS patients takes anaplastic large cell morphology. *Mod Pathol*. 2000;13:77-85.
36. Ely SA, Powers J, Lewis D, et al. Kaposi's sarcoma-associated herpesvirus-positive primary effusion lymphoma arising in the subarachnoid space. *Hum Pathol*. 1999;30:981-984.
37. Katano H, Sato Y, Kurata T, et al. High expression of HHV-8-encoded ORF73 protein in spindle-shape cells of Kaposi's sarcoma. *Am J Pathol*. 1999;155:47-52.
38. Said JW, Shintaku IP, Asou H, et al. Herpesvirus 8 inclusions in primary effusion lymphoma: report of a unique case with T-cell phenotype. *Archiv Pathol Lab Med*. 1999;123:257-260.
39. Cheuk W, Wong KO, Wong CS, et al. Immunostaining for human herpesvirus 8 latent nuclear antigen-1 helps distinguish Kaposi sarcoma from its mimickers. *Am J Clin Pathol*. 2004;121:335-342.
40. Robin YM, Guillou L, Michels JJ, et al. Human herpesvirus 8 immunostaining. A sensitive and specific method for diagnosing Kaposi sarcoma in paraffin-embedded sections. *Am J Clin Pathol*. 2004;121:330-334.
41. Wada DA, Perkins SL, Tripp S, et al. Human herpesvirus 8 and iron staining are useful in differentiating Kaposi sarcoma from interstitial granuloma annulare. *Am J Clin Pathol*. 2007;127:263-270.
42. Bryant-Greenwood P, Sorbara L, Filie AC, et al. Infection of mesothelial cells with human herpesvirus 8 in human immunodeficiency virus-infected patients with Kaposi sarcoma, Castleman disease, and recurrent pleural effusions. *Mod Pathol*. 2003;16:145-153.
43. de la Hoz RE, Stephens G, Sherlock C. Diagnosis and treatment approaches to CMV infections in adult patients. *J Clin Virol*. 2002;25(suppl 2):S1-S12.
44. Bronsther O, Makowka L, Jaffe R. The occurrence of cytomegalovirus hepatitis in liver transplant patients. *J Med Virol*. 1988;24:423-434.
45. Drummer JS, White LT, Ho M, et al. Morbidity of cytomegalovirus infection in recipients of heart or heart-lung transplant who receive cyclosporine. *J Infect Dis*. 1985;152:1182-1191.
46. Anwar F, Erice A, Jessurun J. Are there cytopathic features associated with cytomegalovirus infection predictive of resistance to antiviral therapy? *Ann Diag Pathol*. 1999;3:19-22.
47. Cote L, Drouet E, Bissuel F, et al. Diagnostic value of amplification of human cytomegalovirus DNA from gastrointestinal biopsies from human immunodeficiency virus-infected patients. *J Clin Microbiol*. 1993;31:2066-2069.
48. Rawlinson WD. Broadsheet number 50: Diagnosis of human cytomegalovirus infection and disease. *Pathology*. 1999;31:109-115.
49. Sheehan MM, Coker R, Coleman DV. Detection of cytomegalovirus (CMV) in HIV+ patients: comparison of cytomorphology, immunohistochemistry and in situ hybridization. *Cytopath*. 1998;9:29-37.
50. Kutza AS, Muhl E, Hackstein H, et al. High incidence of active cytomegalovirus infection among septic patients. *Clin Infect Dis*. 1998;26:1076-1082.
51. Saetta A, Agapitos E, Davaris PS. Determination of CMV placentitis: Diagnostic application of the polymerase chain reaction. *Virchows Arch*. 1998;432:159-162.
52. Solans EP, Yong S, Husain AN, et al. Bronchioloalveolar lavage in the diagnosis of CMV pneumonitis in lung transplant recipients: an immunocytochemical study. *Diagn Cytopath*. 1997;16:350-352.
53. Nebuloni M, Pellegrinelli A, Ferri A, et al. Etiology of microglial nodules in brains of patients with acquired immunodeficiency syndrome. *J Neurovirol*. 2000;6:46-50.

54. Rimsza LM, Vela EE, Frutiger YM, et al. Rapid automated combined in situ hybridization and immunohistochemistry for sensitive detection of cytomegalovirus in paraffin-embedded tissue biopsies. *Am J Clin Pathol*. 1996;106:544-548.
55. Kandiel A, Lashner B. Cytomegalovirus colitis complicating inflammatory bowel disease. *Am J Gastroenterol*. 2006;101:2857-2865.
56. Kambham N, Vij R, Cartwright CA, et al. Cytomegalovirus infection in steroid-refractory ulcerative colitis. A case-control study. *Am J Surg Pathol*. 2004;28:365-373.
57. Ribalta T, Martinez AJ, Jares P, et al. Presence of occult cytomegalovirus infection in the brain after orthotopic liver transplantation. An autopsy study of 83 cases. *Virchows Arch*. 2002;440:166-171.
58. Cruz-Spano L, Lima-Pereira FE, Gomes da Silva-Basso N, et al. Human cytomegalovirus infection and abortion: an immunohistochemical study. *Med Sci Monit*. 2002;8:BR230-BR235.
59. Seehofer D, Rayes N, Tullius SG, et al. CMV hepatitis after liver transplantation: incidence, clinical course, and long term follow-up. *Liver Transp*. 2002;8:1138-1146.
60. Lautenschlager I, Hockerstedt K, Taskinen E. Histologic findings associated with CMV infection in liver transplantation. *Transplant Proc*. 2003;35:819.
61. Lamps LW, Pinson CW, Raiford DS, et al. The significance of microabscesses in liver transplant biopsies: a clinicopathological study. *Hepatology*. 1998;28:1532-1537.
62. Barkholt LM, Ehrnst A, Veress B. Clinical use of immunohistopathologic methods for the diagnosis of cytomegalovirus hepatitis in human liver allograft biopsy specimens. *Scand J Gastroenterol*. 1994;29:553-560.
63. Colina F, Jucá NT, Moreno E, et al. Histological diagnosis of cytomegalovirus hepatitis in liver allografts. *J Clin Pathol*. 1995;48:351-357.
64. Lones MA, Shintaku IP, Weiss LM, et al. Posttransplant lymphoproliferative disorder in liver allograft biopsies: a comparison of three methods for the demonstration of Epstein Barr virus. *Hum Pathol*. 1997;28:533-539.
65. Challoner PB, Smith KT, Parker JD, et al. Plaque-associated expression of human herpesvirus 6 in multiple sclerosis. *Proc Natl Acad Sci U S A*. 1995;92:7440-7444.
66. Anderson J. Epstein-Barr virus and Hodgkin's lymphoma. *HERPES*. 2006;13:12-16.
67. Flomenberg P, Babbitt J, Drobyski WR, et al. Increasing incidence of adenovirus disease in bone marrow transplant recipients. *J Infect Dis*. 1994;169:775-781.
68. Strickler JG, Singleton TP, Copenhaver GM, et al. Adenovirus in the gastrointestinal tracts of immunosuppressed patients. *Am J Clin Pathol*. 1992;97:555-558.
69. Yi ES, Powell HC. Adenovirus infection of the duodenum in an AIDS patient: an ultrastructural study. *Ultrastruct Pathol*. 1994;18:549-551.
70. Yan Z, Nguyen S, Poles M, et al. Adenovirus colitis in human immunodeficiency virus infection: an underdiagnosed entity. *Am J Surg Pathol*. 1998;22:1101-1106.
71. Dombrowski F, Eis-Hubinger AM, Ackermann T, et al. Adenovirus-induced liver necrosis in a case of AIDS. *Virchows Archiv*. 1997;431:469-472.
72. Simsir A, Greenebaum E, Nuovo G, et al. Late fatal adenovirus pneumonitis in a lung transplant recipient. *Transplantation*. 1998;65:592-594.
73. Saad RS, Demetris AJ, Lee RG, et al. Adenovirus hepatitis in the adult allograft liver. *Transplantation*. 1997;64:1483-1485.
74. Ohori NP, Michaels MG, Jaffe R, et al. Adenovirus pneumonia in lung transplant recipients. *Hum Pathol*. 1995;26:1073-1079.
75. Marc J, Dieterich DT. The histopathology of 103 consecutive colonoscopy biopsies from 82 symptomatic patients with acquired immunodeficiency syndrome. *Arch Pathol Lab Med*. 2001;125:1042-1046.
76. El-Mahallawy HA, Mansour T, El-Din SE, et al. Parvovirus B19 infection as a cause of anemia in pediatric acute lymphoblastic leukaemia during maintenance chemotherapy. *J Pediatr Hematol Oncol*. 2004;26:403-406.
77. Brown KE, Young NS. Parvovirus B19 infection and hematopoiesis. *Blood Rev*. 1995;9:176-182.
78. Jordan JA, Penchansky L. Diagnosis of human parvovirus B19-induced anemia: correlation of bone marrow morphology with molecular diagnosis using PCR and immunohistochemistry. *Cell Vision*. 1995;2:279-282.
79. Morey AL, O'Neil HJ, Coyle PV, et al. Immunohistological detection of human parvovirus B19 in formalin-fixed, paraffin-embedded tissues. *J Pathol*. 1992;166:105-108.
80. Puvion-Dutilleul F, Puvion E. Human parvovirus B19 as a causative agent for rheumatoid arthritis. *Proc Nat Acad Sci U S A*. 1998;95:8227-8232.
81. Yufu Y, Matsumoto M, Miyamura T, et al. Parvovirus B19-associated haemophagocytic syndrome with lymphadenopathy resembling histiocytic necrotizing lymphadenitis (Kikuchi's disease). *Br J Haematol*. 1997;96:868-871.
82. Vadlamudi G, Rezuke N, Ross JW, et al. The use of monoclonal antibody R92F6 and polymerase chain reaction to confirm the presence of parvovirus B19 in bone marrow specimens of patients with acquired immunodeficiency syndrome. *Arch Pathol Lab Med*. 1999;123:768-773.
83. Wright C, Hinchliffe SA, Taylor C. Fetal pathology in intrauterine death due to parvovirus B19 infection. *Br J Obstet Gynaecol*. 1996;103:133-136.
84. Essary LR, Vnencak-Jones CL, Manning SS, et al. Frequency of parvovirus B19 infection in nonimmune hydrops fetalis and utility of three diagnostic methods. *Hum Pathol*. 1998;29:696-701.
85. Monath TP, Ballinger ME, Miller BR, et al. Detection of yellow fever viral RNA by nucleic acid hybridization and viral antigen by immunohistochemistry in fixed human liver. *Am J Trop Med Hyg*. 1989;40:663-668.
86. De Brito T, Siqueira SA, Santos RT, et al. Human fatal yellow fever. Immunohistochemical detection of viral antigens in the liver, kidney and heart. *Pathol Res Pract*. 1992;188:177-181.
87. Hall WC, Crowell TP, Watts DM, et al. Demonstration of yellow fever and dengue antigens in formalin-fixed paraffin-embedded human liver by immunohistochemical analysis. *Am J Trop Med Hyg*. 1991;45:408-417.
88. Ramos C, Sanchez G, Pando RH, et al. Dengue virus in the brain of a fatal case of hemorrhagic dengue fever. *J Neurovirol*. 1998;4:465-468.
89. Burt FJ, Swanepoel R, Shieh W-J, et al. Immunohistochemical and in situ localization of Crimean-Congo hemorrhagic fever (CCHF) virus in human tissues and implications for CCHF pathogenesis. *Arch Pathol Lab Med*. 1997;121:839-846.
90. Maiztegui JI, Laguens RP, Cossio PM, et al. Ultrastructural and immunohistochemical studies in five cases of Argentine hemorrhagic fever. *J Infect Dis*. 1975;132:35-53.
91. Hall WC, Geisbert TW, Huggins JW, et al. Experimental infection of guinea pigs with Venezuelan hemorrhagic fever virus (Guanarito): a model of human disease. *Am J Trop Med Hyg*. 1996;55:81-88.
92. Geisbert TW, Jaax NK. Marburg hemorrhagic fever: report of a case studied by immunohistochemistry and electron microscopy. *Ultrastruct Pathol*. 1998;22:3-17.
93. Zaki SR, Shieh W-J, Greer PW, et al. A novel immunohistochemical assay for the detection of Ebola virus in skin: implications for diagnosis, spread, and surveillance of Ebola hemorrhagic fever. *J Infect Dis*. 1999;179(suppl 1):S36-S37.
94. Ksiazek TG, Rollin PE, Williams AJ, et al. Clinical virology of Ebola hemorrhagic fever (EHF): virus, virus antigen, and IgG and IgM antibody findings among EHF patients in Kikwit, Democratic Republic of Congo. *J Infect Dis*. 1999;179:S177-S187.
95. Wyers M, Formenty P, Cherel Y, et al. Histopathological and immunohistochemical studies of lesions associated with Ebola virus in a naturally infected chimpanzee. *J Infect Dis*. 1999;179(suppl 1):S54-S59.
96. Nickeleit V, Hirsch HH, Zeiler M, et al. BK-virus nephropathy in renal transplants-tubular necrosis, MHC-class II expression and rejection in a puzzling game. *Nephrol Dial Transplant*. 2000;15:324-332.
97. White LH, Casian A, Hilton R, et al. BK virus nephropathy in renal transplant patients in London. *Transplantation*. 2008;85:1008-1015.
98. Drachenberg CB, Papadimitriou JC, Ramos E. Histologic versus molecular diagnosis of BK polyomavirus-associated nephropathy: a shifting paradigm? *Clin J Am Soc Nephrol*. 2006;1:374-379.

99. Vogler C, Wang Y, Brink DS, et al. Renal pathology in the pediatric transplant patient. *Adv Anat Pathol*. 2007;14:202-216.
100. Dall A, Hariharan S. BK virus nephritis after renal transplantation. *Clin J Am Soc Nephrol*. 2008;3(suppl 2):S68-S75.
101. Latif S, Zaman F, Veeramachaneni R, et al. BK polyomavirus in renal transplants: role of electron microscopy and immunostaining in detecting early infection. *Ultrastruct Pathol*. 2007;31:199-207.
102. Nebuloni M, Tosoni A, Boldorini R, et al. BK virus renal infection in a patient with the acquired immunodeficiency syndrome. *Arch Pathol Lab Med*. 1999;123:807-811.
103. Elli A, Banfi G, Battista-Fogazzi G, et al. BK polyomavirus interstitial nephritis in a renal transplant patient with no previous acute rejection episodes. *J Nephrol*. 2002;15:313-316.
104. Jochum W, Weber T, Frye S, et al. Detection of JC virus by anti-VP1 immunohistochemistry in brains with progressive multifocal leukoencephalopathy. *Acta Neuropathol*. 1997;94:226-231.
105. Chima SC, Agostini HT, Ryschlkeewitsch CF, et al. Progressive multifocal leukoencephalopathy and JC virus genotypes in West African patients with acquired immunodeficiency syndrome. A pathologic and DNA sequence analysis of 4 cases. *Arch Pathol Lab Med*. 1999;123:395-403.
106. Aoki N, Mori M, Kato K, et al. Antibody against synthetic multiple antigen peptides (MAP) of JC virus capsid protein (VP1) without cross reaction to BK virus: a diagnostic tool for progressive multifocal leukoencephalopathy. *Neurosci Lett*. 1996;205:111-114.
107. Silver SA, Arthur RR, Rozan YS, et al. Diagnosis of progressive multifocal leukoencephalopathy by stereotactic brain biopsy utilizing immunohistochemistry and the polymerase chain reaction. *Acta Cytol*. 1995;39:35-44.
108. Guarner J, Shieh WJ, Dawson J, et al. Immunohistochemical and in situ hybridization studies of influenza A virus infection in human lungs. *Am J Clin Pathol*. 2000;114:227-233.
109. Guarner J, Paddock CD, Shieh WJ, et al. Histopathologic and immunohistochemical features of fatal influenza virus infection in children during the 2003-2004 season. *Clin Infect Dis*. 2006;43:132-140.
110. Nielson KA, Yunis EJ. Demonstration of respiratory syncytial virus in an autopsy series. *Pediatr Pathol*. 1990;10:491-502.
111. Wright C, Oliver KC, Fenwick FI, et al. A monoclonal antibody pool for routine immunohistochemical detection of human respiratory syncytial virus antigens in formalin-fixed, paraffin-embedded tissue. *J Pathol*. 1997;182:238-244.
112. Jogai S, Radotra BD, Banerjee AK. Immunohistochemical study of human rabies. *Neuropathology*. 2000;20:197-203.
113. Jogai S, Radotra BD, Banerjee AK. Rabies viral antigen in extracranial organs: a postmortem study. *Neuropathol Appl Neurobiol*. 2002;28:334.
114. Warner CK, Zaki SR, Shieh WJ, et al. Laboratory investigation of humans from vampire bat rabies in Peru. *Am J Trop Med Hyg*. 1999;60:502-507.
115. Sinchaisri TA, Nagata T, Yoshikawa Y, et al. Immunohistochemical and histopathological study of experimental rabies infection in mice. *J Vet Med Sci*. 1992;54:409-416.
116. Jackson AC, Ye H, Phelan CC, et al. Extraneural organ involvement in human rabies. *Lab Invest*. 1999;79:945-951.
117. Yousef GE, Mann GF, Brown IN, et al. Clinical and research application of an enterovirus group-reactive monoclonal antibody. *Intervirol*. 1987;28:199-205.
118. Hohenadl C, Klingel K, Rieger P, et al. Investigation of the coxsackievirus B3 nonstructural proteins 2B, 2C, and 3AB: generation of specific polyclonal antisera and detection of replicating virus in infected tissue. *J Virol Methods*. 1994;47:279-295.
119. Zhang H, Li Y, Peng T, et al. Localization of enteroviral antigen in myocardium and other tissues from patients with heart muscle disease by an improved immunohistochemical technique. *J Histochem Cytochem*. 2000;48:579-584.
120. Li Y, Bourlet T, Andreoletti L, et al. Enteroviral capsid protein VP1 is present in myocardial tissues from some patients with myocarditis or dilated cardiomyopathy. *Circulation*. 2000;101:231-234.
121. Del Piero F, Wilkins PA, Dubovi EJ, et al. Clinical, pathologic, immunohistochemical, and virologic findings of eastern equine encephalitis in two horses. *Vet Pathol*. 2001;38:451-456.
122. Patterson JS, Maes RK, Mullaney TP, et al. Immunohistochemical diagnosis of eastern equine encephalomyelitis. *J Vet Diag Invest*. 1996;8:156-160.
123. Garen PD, Tsai TF, Powers JM. Human eastern equine encephalitis: immunohistochemistry and ultrastructure. *Mod Pathol*. 1999;12:646-652.
124. Tatti KM, Gentsch J, Shieh WJ, et al. Molecular and immunological methods to detect rotavirus in formalin-fixed tissue. *J Virol Methods*. 2002;105:305-319.
125. Morrison C, Gilson T, Nuovo GJ, et al. Histologic distribution of fatal rotaviral infection: an immunohistochemical and reverse transcriptase in situ polymerase chain reaction analysis. *Hum Pathol*. 2001;32:216-221.
126. Cioc AM, Nuovo GJ. Histologic and in situ viral findings in the myocardium in cases of sudden, unexpected death. *Mod Pathol*. 2001;15:914-922.
127. Toulaymant M, Marconi S, Garb J, et al. Endoscopic biopsy pathology of Helicobacter pylori gastritis. *Arch Pathol Lab Med*. 1999;123:778-781.
128. El-Zimaity HMT, Graham DY, Al-Assis MT, et al. Interobserver variation in the histopathological assessment of Helicobacter pylori gastritis. *Hum Pathol*. 1996;27:35-41.
129. Marcio L, Angelucci D, Grossi L, et al. Anti-Helicobacter pylori specific antibody immunohistochemistry improves the diagnostic accuracy of Helicobacter pylori in biopsy specimen from patients treated with triple therapy. *Am J Gastroenterol*. 1998;93:223-226.
130. Rotimi O, Cairns A, Gray S, et al. Histological identification of Helicobacter pylori: comparison of staining methods. *J Clin Pathol*. 2000;53:756-759.
131. Goldstein NS. Chronic inactive gastritis and coccoid Helicobacter pylori in patients treated for gastroesophageal reflux disease or with H. pylori eradication therapy. *Am J Clin Pathol*. 2002;118:719-726.
132. Eshun JK, Black DD, Casteel HB. Comparison of immunohistochemistry and silver stain for the diagnosis of pediatric Helicobacter pylori infection in urease-negative gastric biopsies. *Ped Devel Pathol*. 2001;4:82-88.
133. Lepidi H, Fenollar F, Dumler JS, et al. Cardiac valves in patients with Whipple endocarditis: microbiological, molecular, quantitative histologic, and immunohistochemical studies of 5 patients. *J Infect Dis*. 2004;190:935-945.
134. Houpikian P, Raoult D. Diagnostic methods: current best practices and guidelines for identification of difficult-to-culture pathogens in infective endocarditis. *Infect Dis Clin N Am*. 2007;16:377-392.
135. Baisden BL, Lepidi H, Raoult D, et al. Diagnosis of Whipple disease by immunohistochemical analysis. A sensitive and specific method for the detection of Tropheryma whipplei (the Whipple bacillus) in paraffin-embedded tissue. *Am J Clin Pathol*. 2002;118:742-748.
136. Lepidi H, Fenollar F, Gerolami R, et al. Whipple disease: Immunospecific and quantitative immunohistochemical study of intestinal biopsy specimens. *Hum Pathol*. 2003;34:589-596.
137. Lepidi H, Costedoat N, Piette JC, et al. Immunohistological detection of Tropheryma whipplei (Whipple bacillus) in lymph nodes. *Am J Med*. 2002;113:334-336.
138. Dumler JS, Walker DH. Diagnostic tests for Rocky Mountain spotted fever and other rickettsial diseases. *Dermat Clin*. 1994;12:25-36.
139. Parola P, Paddock CD, Raoult D. Tick-borne rickettsioses around the world: Emerging diseases challenging old concepts. *Clin Microbiol Rev*. 2005;18:719-756.
140. Walker DH, Burday MS, Folds JD. Laboratory diagnosis of Rocky Mountain spotted fever. *South Med J*. 1980;73:1443-1447.
141. Paddock CD, Greer PW, Ferebee TL, et al. Hidden mortality attributable to Rocky Mountain spotted fever: immunohistochemical detection of fatal, serologically unconfirmed disease. *J Infect Dis*. 1999;179:1469-1476.
142. Kaplowitz LG, Lange JV, Fischer JJ, Walker DH. Correlation of rickettsial titers, circulating endotoxin, and clinical features in Rocky Mountain spotted fever. *Arch Intern Med*. 1983;143:1149-1151.

143. Dumler JS, Gage WR, Pettis GL, et al. Rapid immunoperoxidase demonstration of Rickettsia rickettsii in fixed cutaneous specimens from patients with Rocky Mountain spotted fever. *Am J Clin Pathol*. 1990;93:410-414.
144. Chapman AS, Bakken JS, Folk SM, et al. Diagnosis and management of tickborne rickettsial diseases: Rocky Mountain spotted fever, ehrlichiosis, and anaplasmosis—United States: A practical guide for physicians and other health-care and public health professionals. *MMWR Recomm Rep*. 2006;55:1-27.
145. Spach DH, Koehler JE. Bartonella-associated infections. *Infect Dis Clin North Am*. 1998;12:137-155.
146. Lepidi H, Durack DT, Raoult D. Diagnostic methods: Current best practices and guidelines for histologic evaluation in infective endocarditis. *Infect Dis Clin North Am*. 2002;16:339-361.
147. Albrich WC, Kraft C, Fisk T, et al. A mechanic with a bad valve: Blood-culture-negative endocarditis. *Lancet Infect Dis*. 2004;4:777-784.
148. Lepidi H, Fournier PE, Raoult D. Quantitative analysis of valvular lesions during Bartonella endocarditis. *Am J Clin Pathol*. 2000;114:880-889.
149. Baorto E, Payne RM, Slater LN, et al. Culture-negative endocarditis caused by Bartonella henselae. *J Pediatr*. 1998;132:1051-1054.
150. Reed JA, Brigati DJ, Flynn SD, et al. Immunohistochemical identification of Rochalimaea henselae in bacillary (epithelioid) angiomatosis, parenchymal bacillary peliosis, and persistent fever with bacteremia. *Am J Surg Pathol*. 1992;16:650-657.
151. Min KW, Reed JA, Welch DF, et al. Morphologically variable bacilli of cat scratch disease are identified by immunohistochemical labeling with antibodies to Rochalimaea henselae. *Am J Clin Pathol*. 1994;101:607-610.
152. Daybell D, Paddock CD, Zaki SR, et al. Disseminated infection with Bartonella henselae as a cause of spontaneous splenic rupture. *Clin Infect Dis*. 2004;39:21-24.
153. Buffet M, Grange PA, Gerhardt P, et al. Diagnosing Treponema pallidum in secondary syphilis by PCR and immunohistochemistry. *J Invest Dermatol*. 2007;127:2345-2350.
154. Jethwa HS, Schmitz JL, Dallabetta G, et al. Comparison of molecular and microscopic techniques for detection of Treponema pallidum in genital ulcers. *J Clin Microbiol*. 1995;33:180-183.
155. Kingston AA, Vujevich J, Shapiro M, et al. Seronegative secondary syphilis in 2 patients coinfected with human immunodeficiency virus. *Arch Dermatol*. 2005;141:431-433.
156. Hoang MP, High WA, Molberg KH. Secondary syphilis: A histologic and immunohistochemical evaluation. *J Cutan Pathol*. 2004;31:595-599.
157. Guarner J, Greer PW, Bartlett J, et al. Congenital syphilis in a newborn: an immunopathologic study. *Mod Pathol*. 1999;12:82-87.
158. Ulrichs T, Lefmann K, Reich M, et al. Modified immunohistological staining allows detection of Ziehl-Neelsen-negative Mycobacterium tuberculosis organisms and their precise localization in human tissues. *J Pathol*. 2005;205:633-640.
159. Daniel TM. The rapid diagnosis of tuberculosis: A selective review. *J Lab Clin Med*. 1990;116:277-282.
160. Carabias E, Palenque R, Serrano JM, et al. Evaluation of an immunohistochemical test with polyclonal antibodies raised against mycobacteria used in formalin-fixed tissue compared with mycobacterial specific culture. *APMIS*. 1998;106:385-388.
161. Mustafa T, Hg Wiker, Mfinanga SGM, et al. Immunohistochemistry using a Mycobacterium tuberculosis complex specific antibody for improved diagnosis of tuberculosis lymphadenitis. *Mod Pathol*. 2006;19:1606-1614.
162. Zaki SR, Shieh WJ, and the Epidemic Working Group. Leptospirosis associated with an outbreak of acute febrile illness and pulmonary hemorrhage, Nicaragua 1995. *Lancet*. 1996;347:535-536.
163. Trevejo RT, Rigau-Pérez JG, Ashford DA, et al. Epidemic leptospirosis associated with pulmonary hemorrhage-Nicaragua. *J Infect Dis*. 1995;1998(178):1457-1463.
164. Guarner J, Shieh WJ, Morgan J, et al. Leptospirosis mimicking acute cholecystitis among athletes participating in a triathlon. *Hum Pathol*. 2001;32:750-752.
165. Nicodemo AC, Duarte MI, Alves VA, et al. Lung lesions in human leptospirosis: microscopic, immunohistochemical, and ultrastructural features related to thrombocytopenia. *Am J Trop Med Hyg*. 1997;56:181-187.
166. Lebech AM, Clemmensen O, Hansen K. Comparison of in vitro, immunohistochemical staining, and PCR for detection of Borrelia burgdorferi in tissue from experimentally infected animals. *J Clin Microbiol*. 1995;33:2328-2333.
167. Aberer E, Kersten A, Klade H, et al. Heterogeneity of Borrelia burgdorferi in the skin. *Am J Dermatopathol*. 1996;18:571-579.
168. Brouqui P, Raoult D. Endocarditis due to rare and fastidious bacteria. *Clin Microbiol Rev*. 2001;14:177-207.
169. Brouqui P, Dumler JS, Raoult D. Immunohistologic demonstration of Coxiella burnetii in the valves of patients with Q fever endocarditis. *Am J Med*. 1994;97:451-458.
170. Guarner J, Packard MM, Nolte KB, et al. Usefulness of immunohistochemical diagnosis of Streptococcus pneumoniae in formalin-fixed, paraffin-embedded specimens compared with culture and gram stain techniques. *Am J Clin Pathol*. 2007;127:617-618.
171. Guarner J, Bartlett J, Reagan S, et al. Immunohistochemical evidence of Clostridium sp, Staphylococcus aureus, and group A Streptococcus in severe soft tissue infections related to injection drug use. *Hum Pathol*. 2006;37:1482-1488.
172. Guarner J, Sumner J, Paddock CD, et al. Diagnosis of invasive group A streptococcal infections by using immunohistochemical and molecular assays. *Am J Clin Pathol*. 2006;126:148-155.
173. Terpstra WJ, Groeneveld K, Eijk PP, et al. Comparison of two nonculture techniques for detection of Hemophilus influenzae in sputum: In situ hybridization and immunoperoxidase staining with monoclonal antibodies. *Chest*. 1988;94:126S.
174. Groeneveld K, van Alphen L, van Ketel RJ, et al. Nonculture detection of Haemophilus influenzae in sputum with monoclonal antibodies specific for outer membrane lipoprotein P6. *J Clin Microbiol*. 1989;27:2263.
175. Forsgren J, Samuelson A, Borrelli S, et al. Persistence of nontypeable Haemophilus influenzae in adenoid macrophages: a putative colonization mechanism. *Act Oto-Laryngol*. 1996;116:766-773.
176. Shurbaji MS, Dumler JS, Gage WR, et al. Immunohistochemical detection of chlamydial antigens in association with cystitis. *Am J Pathol*. 1990;93:363.
177. Paukku M, Puolakkainen M, Paavonen T, et al. Plasma cell endometritis is associated with Chlamydia trachomatis infection. *Am J Clin Pathol*. 1999;112:211-215.
178. Naas J, Gnarpe JA. Demonstration of Chlamydia pneumoniae in tissue by immunohistochemistry. *APMIS*. 1999;107:882-886.
179. Suffin SC, Kaufmann AF, Whitaker B, et al. Legionella pneumophila. Identification in tissue sections by a new immunoenzymatic procedure. *Arch Pathol Lab Med*. 1980;104:283-286.
180. Maruta K, Miyamoto H, Hamada T, et al. Entry and intracellular growth of Legionella dumoffii in alveolar epithelial cells. *Am J Respir Crit Care Med*. 1998;157:1967-1974.
181. Fiore AE, Nuorti JP, Levine OS, et al. Epidemic Legionnaires' disease two decades later: Old sources, new diagnostic methods. *Clin Infect Dis*. 1998;26:426-433.
182. Parkash V, Morotti RA, Joshi V, et al. Immunohistochemical detection of Listeria antigens in the placenta in perinatal listeriosis. *Int J Gynecol Pathol*. 1998;17:343-350.
183. Chiba M, Fukushima T, Koganei K, et al. Listeria monocytogenes in the colon in a case of fulminant ulcerative colitis. *Scand J Gastroenterol*. 1998;33:778-782.
184. Weinstock D, Horton SB, Rowland PH. Rapid diagnosis of Listeria monocytogenes by immunohistochemistry in formalin-fixed brain tissue. *Vet Pathol*. 1995;32:193-195.
185. Pospischil A, Wood RL, Anderson TD. Peroxidase-antiperoxidase and immunogold labeling of Salmonella typhimurium and Salmonella cholerasuis var kunzendorf in tissues of experimentally infected swine. *Am J Vet Res*. 1990;51:619-624.
186. Thygesen P, Martinsen C, Hougen HP, et al. Histologic, cytologic, and bacteriologic examination of experimentally induced Salmonella typhimurium infection in Lewis rats. *Comp Med*. 2000;50:124-132.
187. Walker DH, Feng HM, Ladner S, et al. Immunohistochemical diagnosis of typhus rickettsioses using an anti-lipopolysaccharide monoclonal antibody. *Mod Pathol*. 1997;10:1038-1042.
188. Koss T, Carter EL, Grossman ME, et al. Increased detection of rickettsialpox in a New York City hospital following the anthrax outbreak of 2001: Use of immunohistochemistry for the rapid confirmation of cases in an era of bioterrorism. *Arch Dermatol*. 2003;139:1545-1552.

189. Walker DH, Hudnall SD, Szaniawski WK, et al. Monoclonal antibody-based immunohistochemical diagnosis of rickettsialpox: the macrophage is the principal target. *Mod Pathol*. 1999;12:529-533.
190. Moron CG, Popov VL, Feng HM, et al. Identification of the target cells of Orientia tsutsugamushi in human cases of scrub typhus. *Mod Pathol*. 2001;14:752-759.
191. Schwarz J. The diagnosis of deep mycoses by morphological methods. *Hum Pathol*. 1982;13:519-533.
192. Marques MEA, Coelho KIR, Bacchi CE. Comparison between histochemical and immunohistochemical methods for the diagnosis of sporotrichosis. *J Clin Pathol*. 1992;45:1089-1093.
193. Reed JA, Hemaan BA, Alexander JL, et al. Immunomycology: Rapid and specific immunocytochemical identification of fungi in formalin-fixed, paraffin-embedded material. *J Histochem Cytochem*. 1993;41:1217-1221.
194. Jensen HE, Schonheyder H, Hotchi M, et al. Diagnosis of systemic mycosis by specific immunohistochemical tests. *APMIS*. 1996;104:241-258.
195. Fukuzawa M, Inaba H, Hayama M, et al. Improved detection of medically important fungi by immunoperoxidase staining with polyclonal antibodies. *Virchows Arch*. 1995;427:407-414.
196. Kauffman L. Immunohistologic diagnosis of systemic mycosis: An update. *Eur J Epidemiol*. 1992;8:377-382.
197. Verweij PE, Smedts F, Poot T. Immunoperoxidase staining for identification of Aspergillus species in routinely processed tissue sections. *J Clin Pathol*. 1996;49:798-801.
198. Breier F, Oesterreicher C, Brugger S, et al. Immunohistochemistry with monoclonal antibody against Candida albicans mannan antigen demonstrates cutaneous Candida granulomas as evidence of Candida sepsis in an immunosuppressed host. *Dermatology*. 1997;194:293-296.
199. Marcilla A, Monteagudo C, Mormeneo S, et al. Monoclonal antibody 3H8: A useful tool in the diagnosis of candidiasis. *Microbiol*. 1999;145:695-701.
200. Monteagudo C, Marcilla A, Mormeneo S, et al. Specific immunohistochemical identification of Candida albicans in paraffin-embedded tissue with a new monoclonal antibody (1B12). *Am J Clin Pathol*. 1995;103:130-135.
201. Jarvensivu A, Hietanen J, Rautemaa R, et al. Candida yeast in chronic periodontitis tissues and subgingival microbial biofilms in vivo. *Oral Diseases*. 2004;10:106-112.
202. Williams DW, Jones HS, Allison RT. Immunohistochemical detection of Candida albicans in formalin fixed, paraffin embedded material. *J Clin Pathol*. 1998;51:857-859.
203. Jarvensivu A, Rautemaa R, Sorsa T, et al. Specificity of the monoclonal antibody 3H8 in the immunohistochemical identification of Candida species. *Oral Diseases*. 2006;12:428-433.
204. Kockenberger MB, Canfield PJ, Kozel TR, et al. An immunohistochemical method that differentiates Cryptococcus neoformans varieties and serotypes in formalin-fixed paraffin-embedded tissues. *Med Mycol*. 2001;39:523-533.
205. Tsunemi T, Kamata T, Fumimura Y, et al. Immunohistochemical diagnosis of Cryptococcus neoformans var. gatti infection in chronic meningoencephalitis: The first case in Japan. *Intern Med*. 2001;40:1241-1244.
206. Chamilos G, Luna M, Lewis RE, et al. Invasive fungal infections in patients with hematologic malignancies in a tertiary care cancer center: An autopsy study over a 15-year period (1989-2003). *Haematologica*. 2006;91:986-989.
207. Hayden RT, Isolato PA, Parrett T, et al. In situ hybridization for the differentiation of Aspergillus, Fusarium, and Pseudallescheria species in tissue sections. *Diagn Mol Pathol*. 2003;12:21-26.
208. Rickerts V, Mousset S, Lambrecht E, et al. Comparison of histopathological analysis, culture, and polymerase chain reaction assays to detect invasive mold infections from biopsy specimens. *Clin Infect Dis*. 2007;44:1078-1083.
209. Hope WW, Walsh TJ, Denning DW. Laboratory diagnosis of invasive aspergillosis. *Lancet Infect Dis*. 2005;5:609-622.
210. Tarrand JJ, Lichterfeld M, Warraich I, et al. Diagnosis of invasive septated mold infections. A correlation of microbiological culture and histologic or cytologic examination. *Am J Clin Pathol*. 2003;119:854-858.
211. Choi JK, Mauger J, McGowan KL. Immunohistochemical detection of Aspergillus species in pediatric tissue samples. *Am J Clin Pathol*. 2004;121:18-25.
212. Pierard GE, Arrese-Estrada J, Pierard-Franchimont C, et al. Immunohistochemical expression of galactomannan in the cytoplasm of phagocytic cells during invasive aspergillosis. *Am J Clin Pathol*. 1991;96:373-376.
213. Phillips P, Weiner MH. Invasive aspergillosis diagnosed by monoclonal and polyclonal reagents. *Hum Pathol*. 1987;18:1015-1024.
214. Fenelon LE, Hamilton AJ, Figueroa JI, et al. Production of specific monoclonal antibodies to Aspergillus species and their use in immunohistochemical identification of aspergillosis. *J Clin Microbiol*. 1999;37:1221-1223.
215. Jensen HE, Salonen J, Ekfors TO. The use of immunohistochemistry to improve sensitivity and specificity in the diagnosis of systemic mycoses in patients with haematological malignancies. *J Pathol*. 1997;181:100-105.
216. Wazir JE, Brown I, Martin-Bates E, et al. EB9, a new antibody for the detection of trophozoites of Pneumocystis carinii in bronchoalveolar lavage specimens in AIDS. *J Clin Pathol*. 1994;47:1108-1111.
217. Wazir JE, Macrorie SG, Coleman DV. Evaluation of the sensitivity, specificity, and predictive value of monoclonal antibody 3F6 for the detection of Pneumocystis carinii pneumonia in bronchoalveolar lavage specimens and induced sputum. *Cytopathol*. 1994;5:82-89.
218. Cooper CR, McGinnis MR. Pathology of Penicillium marneffei: An emerging acquired immunodeficiency syndrome-related pathogen. *Arch Lab Pathol Med*. 1997;121:798-804.
219. Chaiwun B, Khunamornpong S, Sirivanichai C, et al. Lymphadenopathy due to Penicillium marneffei infection: Diagnosis by fine needle aspiration cytology. *Mod Pathol*. 2002;15:939-943.
220. Arrese Estrada J, Stynen D, Van Cutsem J, et al. Immunohistochemical identification of Penicillium marneffei by monoclonal antibody. *Int J Dermatol*. 1992;31:410-412.
221. Burke DG, Emancipator SN, Smith MC, et al. Histoplasmosis and kidney disease in patients with AIDS. *Clin Infect Dis*. 1997;25:281-284.
222. Arnold SJ, Kinney MC, McCormick MS, et al. Disseminated toxoplasmosis: Unusual presentations in the immunocompromised host. *Arch Pathol Lab Med*. 1997;121:869-873.
223. Warnke C, Tuazon CU, Kovacs A, et al. Toxoplasma encephalitis in patients with acquired immunodeficiency syndrome: Diagnosis and response to therapy. *Am J Trop Med Hyg*. 1987;36:509.
224. Ganji M, Tan A, Maitar ML, et al. Gastric toxoplasmosis in a patient with acquired immunodeficiency syndrome. A case report and review of the literature. *Arch Pathol Lab Med*. 2003;127:732-734.
225. Hofman V, Brousset P, Mougneau E, et al. Immunostaining of visceral leishmaniasis caused by Leishmania infantum using monoclonal antibody (19-11) to the Leishmania homologue of receptors for activated C-kinase. *Am J Clin Pathol*. 2003;120:567-574.
226. Azadeh B, Sells PG, Ejeckman GC, et al. Localized Leishmania lymphadenitis immunohistochemical studies. *Am J Clin Pathol*. 1994;102:11-15.
227. Kenner JR, Aronson NE, Bratthauer GL, et al. Immunohistochemistry to identify Leishmania parasites in fixed tissues. *J Cutan Pathol*. 1999;26:130-136.
228. Amato VS, Duarte MIS, Nicodemo AC, et al. An evaluation of clinical, serologic, anatomopathologic and immunohistochemical findings for fifteen patients with mucosal leishmaniasis before and after treatment. *Rev Inst Med Trop S Paulo*. 1998;40:23-30.
229. Guarner J, Bartlett J, Shieh WJ, et al. Histopathologic spectrum and immunohistochemical diagnosis of amebic meningoencephalitis. *Mod Pathol*. 2007;20:1230-1237.
230. Bonnin A, Petrella T, Dubremetz JF, et al. Histopathologic methods for diagnosis of cryptosporidiosis using monoclonal antibodies. *Eur J Clin Microbiol Infect Dis*. 1990;9:664-665.
231. Perez de Suarez E, Perez-Schael I, Perozo-Ruggeri G, et al. Immunocytochemical detection of Entamoeba histolytica. *Trans R Soc Trop Med Hyg*. 1987;81:624-626.

232. Guarner J, Bartlett J, Zaki SR, et al. Mouse model for Chagas disease: Immunohistochemical distribution of different stages of Trypanosoma cruzi in tissues throughout infection. *Am J Trop Med Hyg*. 2001;65:152-158.
233. Anez N, Carrasco H, Parada H, et al. Myocardial parasite persistence in chronic chagasic patients. *Am J Trop Med Hyg*. 1999;60:726-732.
234. Reis MM, Higuchi Mde L, Benvenuti LA, et al. An in situ quantitative immunohistochemical study of cytokines and IL-2R+ in chronic human chagasic myocarditis: correlation with the presence of myocardial Trypanosoma cruzi antigens. *Clin Immunol Immunopathol*. 1997;83:165-172.
235. Torres-Velez FJ, Nace EK, Won KY, et al. Development of an immunohistochemical assay for the detection of babesiosis in formalin-fixed, paraffin-embedded tissue samples. *Am J Clin Pathol*. 2003;120:833-838.
236. Sanad MM, Darwish RA, Nasr ME, et al. Giardia lamblia and chronic gastritis. *J Egypt Soc Parasitol*. 1996;26:481-495.
237. Genrich GL, Guarner J, Paddock CD, et al. Fatal malaria infection in travelers: Novel immunohistochemical assays for the detection of Plasmodium falciparum in tissues and implications for pathogenesis. *Am J Trop Med Hyg*. 2007;76:251-259.
238. Institute of Medicine. *Emerging infections: microbial threats to health in the United States*. Washington, DC: National Academy Press; 1992.
239. CDC. Preventing Emerging Infectious Diseases: A Strategy for the 21st Century. Overview of the Updated CDC Plan. *MMWR Recomm Rep*. 1998;47:1-14.
240. Perkins BA, Flood JM, Danila R, et al. Unexplained deaths due to possibly infectious causes in the United States: Defining the problem and designing surveillance and laboratory approaches. *Emerg Infect Dis*. 1998;2:47-53.
241. Houpikina P, Raoult D. Traditional and molecular techniques for the study of emerging bacterial diseases: One laboratory's perspective. *Emerg Infect Dis*. 2002;8:122-131.
242. Khan AS, Khabbaz RF, Armstrong LR, et al. Hantavirus pulmonary syndrome: The first 100 US cases. *J Infect Dis*. 1996;173:1297-1303.
243. Moolenaar RL, Dalton C, Lipman HB, et al. Clinical features that differentiate hantavirus pulmonary syndrome from three other acute respiratory illnesses. *Clin Infect Dis*. 1995;21:643-649.
244. Nolte KB, Feddersen RM, Foucar K, et al. Hantavirus pulmonary syndrome in the United States: A pathological description of a disease caused by a new agent. *Hum Pathol*. 1995;26:110-120.
245. Zaki SR, Greer PW, Coffield LM, et al. Hantavirus pulmonary syndrome. Pathogenesis of an emerging infectious disease. *Am J Pathol*. 1995;146:552-579.
246. Zaki SR, Khan AS, Goodman RA, et al. Retrospective diagnosis of hantavirus pulmonary syndrome, 1978-1993. Implications for emerging infectious diseases. *Arch Pathol Lab Med*. 1996;120:134-139.
247. Shieh WJ, Guarner J, Layton M, et al. The role of pathology in an investigation of an outbreak of West Nile encephalitis in New York, 1999. *Emerg Infect Dis*. 2000;6:370-372.
248. Cushing MM, Brat DJ, Mosunjac MI, et al. Fatal West Nile virus encephalitis in a renal transplant recipient. *Am J Clin Pathol*. 2004;121:26-31.
249. Petersen RL, Roehrig JT, Hughes JM. West Nile virus encephalitis. *N Engl J Med*. 2002;347:1225-1226.
250. Sampson BA, Ambrosi C, Charlot A, et al. The pathology of human West Nile virus infection. *Hum Pathol*. 2000;31:527-532.
251. Guarner J, Shieh WJ, Hunter S, et al. Clinicopathologic study and laboratory diagnosis of 23 cases with West Nile virus encephalomyelitis. *Hum Pathol*. 2004;35:983-990.
252. Lanciotti RS, Kerst AJ, Nasci RS, et al. Rapid detection of West Nile virus from human clinical specimens, field-collected mosquitoes, and avian samples by a TaqMan reverse transcriptase PCR assay. *J Clin Microbiol*. 2000;38:4066-4071.
253. Ho M, Chen ER, Hsu KH, et al. An epidemic of enterovirus 71 infection in Taiwan. Taiwan enterovirus epidemic working group. *N Engl J Med*. 1999;341:929-935.
254. Chan LG, Parashar UD, Lye MS. Deaths of children during an outbreak of hand, foot, and mouth disease in Sarawak, Malaysia: Clinical and pathological characteristics of the disease. *Clin Infect Dis*. 2000;31:678-683.
255. Wong KT, Chua KB, Lam SK. Immunohistochemical detection of infected neurons as a rapid diagnosis of enterovirus 71 encephalomyelitis. *Ann Neurol*. 1999;45:271-272.
256. Yan JJ, Wang JR, Liu CC, et al. An outbreak of enterovirus 71 infection in Taiwan 1998: A comprehensive pathological, virological, and molecular study on a case of fulminant encephalitis. *J Clin Virol*. 2000;17:13-22.
257. Shieh WJ, Jung SM, Hsueh C, et al. Pathologic studies of fatal causes in outbreak of hand, foot, and mouth disease. *Taiwan. Emerg Infect Dis*. 2001;7:146-148.
258. Wong KT, Shieh WJ, Kumar S, et al. Nipah virus infection. Pathology and pathogenesis of an emerging paramyxoviral zoonosis. *Am J Pathol*. 2002;161:2153-2167.
259. Goh KJ, Tan CT, Chew NK, et al. Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. *N Engl J Med*. 2000;342:1229-1235.
260. Chua KB, Bellini WJ, Rota PA, et al. Nipah virus: A recently emergent deadly paramyxovirus. *Science*. 2000;288:1432-1435.
261. Dawson JE, Paddock CD, Warner CK, et al. Tissue diagnosis of Ehrlichia chaffeensis in patients with fatal ehrlichiosis by use of immunohistochemistry, in situ hybridization, and polymerase chain reaction. *Am J Trop Med Hyg*. 2001;65:603-609.
262. Walker DH, Dumler JS. Human monocytic and granulocytic ehrlichiosis. Discovery and diagnosis of emerging tick-borne infections and the critical role of the pathologist. *Arch Pathol Lab Med*. 1997;121:785-791.
263. Paddock CD, Suchard DP, Grumbach KL, et al. Fatal seronegative ehrlichiosis in a patient with HIV infection. *N Engl J Med*. 1993;329:1164-1167.
264. Lepidi H, Bunnell JE, Martin ME, et al. Comparative pathology and immunohistology associated with clinical illness after Ehrlichia phagocytophila-group infections. *Am J Trop Med Hyg*. 2000;62:29-37.
265. Childs JE, Sumner JW, Nicholson WL, et al. Outcome of diagnostic tests using samples from patients with culture-proven human monocytic ehrlichiosis: Implications for surveillance. *J Clin Microbiol*. 1999;37:2997-3000.
266. Sehdev AE, Dumler JS. Hepatic pathology in human monocytic ehrlichiosis. Ehrlichia chaffeensis infection. *Am J Clin Pathol*. 2003;119:859-865.
267. Hooper PT, Russell GM, Selleck PW, et al. Immunohistochemistry in the identification of a number of new diseases in Australia. *Vet Microbiol*. 1999;68:89-93.
268. Williamson MM, Hooper PT, Selleck PW, et al. Experimental Hendra virus infection in pregnant guinea-pigs and fruit bats (Pteropus poliocephalus). *J Comp Pathol*. 2000;122:201-207.
269. Paddock CD, Summer JW, Comer JA, et al. Rickettsia parkeri: A newly recognized cause of spotted fever rickettsiosis in the United States. *Clin Infect Dis*. 2004;38:805-811.
270. Lepidi H, Fournier PE, Raoult D. Histologic features and immunodetection of African tick-bite fever eschar. *Emerg Infect Dis*. 2006;12:1332-1337.
271. Ksiazek TG, Erdman D, Goldsmith CS, et al. A novel coronavirus associated with severe acute respiratory syndrome. *N Engl J Med*. 2003;348:1953-1966.
272. Peiris JS, Lai ST, Poon LL, et al. Coronavirus as a possible cause of severe acute respiratory syndrome. *Lancet*. 2003;361:1319-1325.
273. Nakajima N, Asahi-Ozaki Y, Nagata N, et al. SARS coronavirus-infected cells in lung detected by new in situ hybridization technique. *Jpn J Infect Dis*. 2003;56:139-141.
274. Kuiken T, Fouchier RA, Schutten M, et al. Newly discovered coronavirus as the primary cause of severe acute respiratory syndrome. *Lancet*. 2003;362:263-270.
275. Chong PY, Chui P, Ling AE, et al. Analysis of deaths during the severe acute respiratory syndrome (SARS) epidemic in Singapore: Challenges in determining a SARS diagnosis. *Arch Pathol Lab Med*. 2004;128:195-204.
276. McAuliffe J, Vogel L, Roberts A, et al. Replication of SARS coronavirus administered into the respiratory tract of African green, rhesus and cynomolgus monkeys. *Virology*. 2004;5:8-15.
277. Roberts A, Vogel L, Guarner J, et al. SARS coronavirus infection of golden Syrian hamsters. *J Virol*. 2005;79:503-511.

278. Chen PC, Hsiao CH, Re: To KF, Tong JH, Chan PK, et al. Tissue and cellular tropism of the coronavirus associated with severe acute respiratory syndrome: An in-situ hybridization study of fatal cases. *J Pathol*. 2004;203:729-731.
279. Re: To KF, Tong JH, Chan PK, et al. Tissue and cellular tropism of the coronavirus associated with severe acute respiratory syndrome: An in-situ hybridization study of fatal cases. *J Pathol*. 2004;202:157-163.
280. Chow KC, Hsiao CH, Lin TY, et al. Detection of severe acute respiratory syndrome-associated coronavirus in pneumocytes of the lung. *Am J Clin Pathol*. 2004;121:574-580.
281. Ding Y, He L, Zhang Q, et al. Organ distribution of severe acute respiratory syndrome (SARS) associated coronavirus (SARS-Co-V) in SARS patients: Implications for pathogenesis and virus transmission pathways. *J Pathol*. 2004;203:622-630.
282. Sheih WJ, Cheng-Hsiang H, Paddock CD, et al. Immunohistochemical, in situ hybridization, and ultrastructural localization of SARS-associated coronavirus in lung of a fatal case of severe acute respiratory syndrome in Taiwan. *Hum Pathol*. 2005;36:303-309.
283. Ashford DA, Kaiser RB, Bales ME, et al. Planning against biological terrorism: Lessons from outbreak investigations. *Emerg Infect Dis*. 2003;9:515-519.
284. Inglesby TV, O'Toole T, Henderson DA. Preventing the use of biological weapons: Improving response should prevention fail. *Clin Infect Dis*. 2000;30:926-929.
285. Lillibridge SR, Bell AJ, Roman RS. Thoughts for the new millennium: Bioterrorism. Centers for Disease Control and Prevention bioterrorism preparedness and response. *Am J Infect Control*. 1999;27:463-464.
286. Franz DR, Zajtchuk R. Biological terrorism: Understanding the threat, preparation, and medical response. *Dis Month*. 2000;46:125-190.
287. Guarner J, Zaki SR. Histopathology and immunohistochemistry in the diagnosis of bioterrorism agents. *J Histochem Cytochem*. 2006;54:3-11.
288. Ezzell JW, Abshire TG, Little SF, et al. Identification of Bacillus anthracis by using monoclonal antibodies to cell wall galactose-N-acetylglucosamine polysaccharide. *J Clin Microbiol*. 1990;28:223-231.
289. Shieh WJ, Guarner J, Paddock C, et al. The critical role of pathology in the investigation of bioterrorism-related cutaneous anthrax. *Am J Pathol*. 2003;163:1901-1910.
290. Guarner J, Jernigan JA, Shieh WJ, et al. Pathology and pathogenesis of bioterrorism-related inhalational anthrax. *Am J Pathol*. 2003;163:701-709.
291. Jernigan JA, Stephens DS, Ashford DA, et al. Bioterrorism-related inhalational anthrax: The first 10 cases reported in the United States. *Emerg Infect Dis*. 2001;7:933-944.
292. Jernigan DB, Raghunathan PL, Bell BP, et al. Investigation of bioterrorism-related anthrax, United States, 2001: Epidemiologic findings. *Emerg Infect Dis*. 2002;8:1019-1028.
293. Tatti KM, Greer P, White E, et al. Morphologic, immunologic, and molecular methods to detect Bacillus anthracis in formalin-fixed tissues. *Appl Immunohistochem Mol Morphol*. 2006;14:234-243.
294. Grinberg LM, Abramova FA, Yampolskaya OV, et al. Quantitative pathology of inhalational anthrax I: Quantitative microscopic findings. *Mod Pathol*. 2001;14:482-495.
295. Guarner J, Greer PW, Bartlett J, et al. Immunohistochemical detection of Francisella tularensis in formalin-fixed paraffin-embedded tissue. *Appl Immun Mol Morphol*. 1999;7:122-126.
296. DeBey BM, Andrews GA, Chard-Bergstrom C, et al. Immunohistochemical demonstration of Francisella tularensis in lesions of cats with tularaemia. *J Vet Diagn Invest*. 2002;14:162-164.
297. Guarner J, Shieh WJ, Greer PW, et al. Immunohistochemical detection of Yersinia pestis in formalin-fixed paraffin-embedded tissue. *Am J Clin Pathol*. 2002;117:205-209.
298. Davis KJ, Vogel P, Fritz DL, et al. Bacterial filamentation of Yersinia pestis by β-lactam antibiotics in experimentally infected mice. *Arch Pathol Lab Med*. 1997;121:865-868.
299. Davis KJ, Fritz DL, Pitt ML, et al. Pathology of experimental pneumonic plague produced by fraction 1-positive and fraction 1-negative Yersinia pestis in African green monkeys (Cercopithecus aethiops). *Arch Pathol Lab Med*. 1996;120:156-163.
300. Williams ES, Mills K, Kwiatkowski DR, et al. Plague in black-footed ferret (Mustela nigripes). *J Wild Dis*. 1994;30:581-585.
301. Gabastou JM, Proaño J, Vimos A, et al. An outbreak of plague including cases with probable pneumonic infection, Ecuador, 1998. *Trans R Soc Tro Med Hyg*. 2000;94:387-391.
302. Guarner J, Shieh WJ, Chu M, et al. Persistent Yersinia pestis antigens in ischemic tissues of a patient with septicemic plague. *Hum Pathol*. 2005;36:850-853.
303. Figueroa ME, Rasheed S. Molecular pathology and diagnosis of infectious diseases. *Am J Clin Pathol*. 1991;95:S8-S21.
304. Fredricks DN, Relman DA. Application of polymerase chain reaction to the diagnosis of infectious diseases. *Clin Infect Dis*. 1999;29:475-486;quiz 487-488.
305. McNicol AM, Farquharson MA. In situ hybridization and its diagnostic applications in pathology. *J Pathol*. 1997;182: 250-261.
306. Mothershed EA, Whitney AM. Nucleic acid-based methods for the detection of bacterial pathogens: Present and future considerations for the clinical laboratory. *Clin Chim Acta*. 2006;363:206-220.
307. Procop GW, Wilson M. Infectious disease pathology. *Clin Infect Dis*. 2001;32:1589-1601.
308. Sklar J. DNA hybridization in diagnostic pathology. *Hum Pathol*. 1985;16:654-658.
309. Tang YW, Procop GW, Persing DH. Molecular diagnostics of infectious diseases. *Clin Chem*. 1997;43:2021-2038.
310. Andrade ZR, Garippo AL, Saldiva PH, et al. Immunohistochemical and in situ detection of cytomegalovirus in lung autopsies of children immunocompromised by secondary interstitial pneumonia. *Pathol Res Pract*. 2004;200:25-32.
311. Burt FJ, Swanepoel R, Shieh WJ, et al. Immunohistochemical and in situ localization of Crimean-Congo hemorrhagic fever (CCHF) virus in human tissues and implications for CCHF pathogenesis. *Arch Pathol Lab Med*. 1997;121:839-846.
312. Fredricks DN, Relman DA. Localization of Tropheryma whippelii rRNA in tissues from patients with Whipple's disease. *J Infect Dis*. 2001;183:1229-1237.
313. Gentilomi G, Musiani M, Zerbini M, et al. Double in situ hybridization for detection of herpes simplex virus and cytomegalovirus DNA using non-radioactive probes. *J Histochem Cytochem*. 1992;40:421-425.
314. Gentilomi G, Zerbini M, Musiani M, et al. In situ detection of B19 DNA in bone marrow of immunodeficient patients using a digoxigenin-labelled probe. *Mol Cell Probes*. 1993;7:19-24.
315. Hayden RT, Qian X, Procop GW, et al. In situ hybridization for the identification of filamentous fungi in tissue section. *Diagn Mol Pathol*. 2002;11:119-126.
316. Hayden RT, Qian X, Roberts GD, et al. In situ hybridization for the identification of yeastlike organisms in tissue section. *Diagn Mol Pathol*. 2001;10:15-23.
317. Hulette CM, Downey BT, Burger PC. Progressive multifocal leukoencephalopathy. Diagnosis by in situ hybridization with a biotinylated JC virus DNA probe using an automated Histomatic Code-On slide stainer. *Am J Surg Pathol*. 1991;15: 791-797.
318. Krimmer V, Merkert H, von Eiff C, et al. Detection of Staphylococcus aureus and Staphylococcus epidermidis in clinical samples by 16S rRNA-directed in situ hybridization. *J Clin Microbiol*. 1999;37:2667-2673.
319. Matsuse T, Matsui H, Shu CY, et al. Adenovirus pulmonary infections identified by PCR and in situ hybridisation in bone marrow transplant recipients. *J Clin Pathol*. 1994;47:973-977.
320. Montone KT, Litzky LA. Rapid method for detection of Aspergillus 5S ribosomal RNA using a genus-specific oligonucleotide probe. *Am J Clin Pathol*. 1995;103:48-51.
321. Morey AL, Keeling JW, Porter HJ, et al. Clinical and histopathological features of parvovirus B19 infection in the human fetus. *Br J Obstet Gynaecol*. 1992;99:566-574.
322. Morey AL, Porter HJ, Keeling JW, et al. Non-isotopic in situ hybridisation and immunophenotyping of infected cells in the investigation of human fetal parvovirus infection. *J Clin Pathol*. 1992;45:673-678.
323. Moter A, Göbel UB. Fluorescence in situ hybridization (FISH) for direct visualization of microorganisms. *J Microbiol Methods*. 2000;41:85-112.

324. Musiani M, Zerbini M, Venturoli S, et al. Rapid diagnosis of cytomegalovirus encephalitis in patients with AIDS using in situ hybridisation. *J Clin Pathol*. 1994;47:886-891.
325. Naoumov NV, Daniels HM, Davison F, et al. Identification of hepatitis B virus-DNA in the liver by in situ hybridization using a biotinylated probe. Relation to HBcAg expression and histology. *J Hepatol*. 1993;19:204-210.
326. Porter HJ, Padfield CJ, Peres LC, et al. Adenovirus and intranuclear inclusions in appendices in intussusception. *J Clin Pathol*. 1993;46:154-158.
327. Schmidbauer M, Budka H, Ambros P. Herpes simplex virus (HSV) DNA in microglial nodular brainstem encephalitis. *J Neuropathol Exp Neurol*. 1989;48:645-652.
328. Schmidbauer M, Budka H, Pilz P, et al. Presence, distribution and spread of productive varicella zoster virus infection in nervous tissues. *Brain*. 1992;115(Pt 2):383-398.
329. Thompson CH, Biggs IM, de Zwart-Steffe RT. Detection of molluscum contagiosum virus DNA by in situ hybridization. *Pathology*. 1990;22:181-186.
330. Unger ER. In situ diagnosis of human papillomaviruses. *Clin Lab Med*. 2000;20:289-301.
331. Wu TC, Mann RB, Epstein JI, et al. Abundant expression of EBER1 small nuclear RNA in nasopharyngeal carcinoma. A morphologically distinctive target for detection of Epstein-Barr virus in formalin-fixed paraffin-embedded carcinoma specimens. *Am J Pathol*. 1991;138:1461-1469.
332. Zaki SR, Judd R, Coffield LM, et al. Human papillomavirus infection and anal carcinoma. Retrospective analysis by in situ hybridization and the polymerase chain reaction. *Am J Pathol*. 1992;140:1345-1355.
333. Akhtar N, Ni J, Langston C, et al. PCR diagnosis of viral pneumonitis from fixed-lung tissue in children. *Biochem Mol Med*. 1996;58:66-76.
334. Amaker BH, Chandler Jr FW, Huey LO, et al. Molecular detection of JC virus in embalmed, formalin-fixed, paraffin-embedded brain tissue. *J Forensic Sci*. 1997;42:1157-1159.
335. Beqaj SH, Flesher R, Walker GR, et al. Use of the real-time PCR assay in conjunction with MagNA Pure for the detection of mycobacterial DNA from fixed specimens. *Diagn Mol Pathol*. 2007;16:169-173.
336. Bhatnagar J, Guarner J, Paddock CD, et al. Detection of West Nile virus in formalin-fixed, paraffin-embedded human tissues by RT-PCR: A useful adjunct to conventional tissue-based diagnostic methods. *J Clin Virol*. 2007;38:106-111.
337. Clavel C, Binninger I, Polette M, et al. [Polymerase chain reaction (PCR) and pathology. Technical principles and application]. *Ann Pathol*. 1993;13:88-96.
338. Guarner J, Bhatnagar J, Shieh WJ, et al. Histopathologic, immunohistochemical, and polymerase chain reaction assays in the study of cases with fatal sporadic myocarditis. *Hum Pathol*. 2007;38:1412-1419.
339. Lamps LW, Madhusudhan KT, Greenson JK, et al. The role of Yersinia enterocolitica and Yersinia pseudotuberculosis in granulomatous appendicitis: A histologic and molecular study. *Am J Surg Pathol*. 2001;25:508-515.
340. Paddock CD, Sanden GN, Cherry JD, et al. Pathology and pathogenesis of fatal Bordetella pertussis infection in infants. *Clin Infect Dis*. 2008;47:328-338.
341. Qian X, Jin L, Hayden RT, et al. Diagnosis of cat scratch disease with Bartonella henselae infection in formalin-fixed paraffin-embedded tissues by two different PCR assays. *Diagn Mol Pathol*. 2005;14:146-151.
342. Schild M, Gianinazzi C, Gottstein B, et al. PCR-based diagnosis of Naegleria sp. infection in formalin-fixed and paraffin-embedded brain sections. *J Clin Microbiol*. 2007;45:564-567.
343. Singh HB, Katoch VM, Natrajan M, et al. Improved protocol for PCR detection of Mycobacterium leprae in buffered formalin-fixed skin biopsies. *Int J Lepr Other Mycobact Dis*. 2004;72:175-178.
344. Tatti KM, Wu KH, Sanden GN, et al. Molecular diagnosis of Bordetella pertussis infection by evaluation of formalin-fixed tissue specimens. *J Clin Microbiol*. 2006;44:1074-1076.
345. Wilson DA, Reischl U, Hall GS, et al. Use of partial 16S rRNA gene sequencing for identification of Legionella pneumophila and non-pneumophila Legionella spp. *J Clin Microbiol*. 2007;45:257-258.