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The Modern Autopsy: What to Do if Infection Is Suspected

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Deaths due to infectious diseases are common worldwide. The autopsy, although less frequently performed than previously, is important to our understanding of disease pathogenesis. The autopsy also provides critical information regarding potential disease outbreaks. To optimize the benefits of an autopsy, the pathologist should approach the autopsy with a well-constructed differential diagnosis that provides the framework for appropriate selection of diagnostic specimens and tests. Standard microbiologic cultures, although necessary and important, are often insufficient and must be supplemented by newer molecular methodologies. © 2005 IMSS. Published by Elsevier Inc.

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Introduction

Despite enormous advances in diagnosis and treatment, infectious diseases continue to play a significant role in mortality in the U.S. and worldwide (1). Although autopsy rates in the U.S. have declined, the autopsy remains an important tool that allows the physician to gain additional information into the pathogenesis of disease, to identify clinically unsuspected disease processes, and to correlate premortem clinical diagnosis with postmortem findings (2). The goals of this article are to 1) discuss the role of the modern autopsy in ascertaining cause of death and advancing understanding of the pathogenesis of infectious diseases, 2) identify major clinical syndromes and the appropriate laboratory methods for detecting etiologic agent(s), 3) review the status of microbiologic cultures in the performance of the modern autopsy, 4) discuss the role and potential limitations of molecular pathology in today's autopsy, 5) review methods for protecting personnel in completion of the autopsy, and 6) discuss the role of the pathologist in community/national response to potential acts of bioterrorism.

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Role of the Autopsy in Ascertaining Cause of Death for an Individual

According to the U.S. National Vital Statistics Reports, deaths due to infectious causes are among the leading causes of deaths in whites, blacks, and American Indian populations (1). Whereas these data are derived from death certificate reporting, data derived from individual autopsies continue to provide valuable information regarding cause of death.

For example, a young man was hospitalized for 6 weeks for treatment of acute lymphoblastic leukemia, during which time he developed persistent candidemia and pulmonary aspergillosis. Although infection was recognized antemortem, autopsy findings provided evidence of the extent of disease, amount of tissue destruction, and corroborated antemortem results. The gross and microscopic evidence of right-sided endocarditis explained the refractory candidemia.

Contrast the above scenario with that of a 21-year-old college student who was found dead in her dormitory room, an apparent natural death. Certain infectious diseases progress so rapidly, so as to be initially considered a sudden death. However, even the most fulminant infectious diseases produce symptoms that precede death and are not instantaneous. Discussions with her roommates confirmed she had complained of respiratory symptoms and severe headache the morning of her death. At autopsy, there was gross evidence of purulent meningitis and culture yielded *Neisseria meningitidis*. This case highlights the changing epidemiology of meningitis, which includes an increase in cases

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in college students (3,4). Whereas standard cultures were diagnostic in this case, rapid diagnostic techniques, such as polymerase chain reaction (PCR) may expedite diagnosis and facilitate early public health interventions (5).

Contribution to Disease Pathogenesis

Autopsy findings have served to elucidate the pathogenesis of new diseases. When unusual infections emerged in homosexual men in California in 1991, autopsy data were key to our understanding of what is now called human immunodeficiency virus (HIV) and the acquired immunodeficiency syndrome (AIDS); autopsies studies continue to refine our understanding of disease pathogenesis (6–9).

During the Spring of 1993, the nation was similarly riveted by unexplained deaths occurring in the southwestern U.S., for which numerous scientists collaborated to characterize the clinical syndrome, establish key pathologic findings, and isolate the causative agent, Hantavirus (10–14). More recent examples of new infectious agents/syndromes, for which autopsy data have been invaluable, include West Nile Virus and severe acute respiratory syndrome (SARS) (15–17,18).

Autopsies serve as important epidemiologic tools for identifying potential disease outbreaks and establishing risk factors for specific infections. Through the autopsy, the pathologist may identify a potential disease outbreak, whether due to a common community-acquired pathogen or, more ominously, an unusual pathogen that suggests an act of biologic terrorism. In the 2001 outbreak of inhalational anthrax, autopsy findings were vital to what proved to be an extensive investigation (19).

Given the importance of accurate autopsy data for the aforementioned goals, the pathologist must approach each autopsy with a differential diagnosis based upon antemortem signs and symptoms, results of previous diagnostic studies, and prior microbial isolation, when available. Careful collaboration with the patient's attending physician(s) is essential in constructing the differential diagnosis, the questions to be answered by the postmortem exam, and the appropriate studies needed to accomplish those tasks. When the autopsy is performed for sudden unexplained death, prior studies may be lacking, and much of the differential diagnosis will need to be constructed using relevant past medical history, family interviews, and other historical clues.

Major Clinical Syndromes

An understanding of the potential diagnoses and pathogens provides the basis for appropriate postmortem analysis. A thorough discussion of differential diagnosis in infectious diseases is beyond the scope of this article; the reader is referred to several authoritative resources on this topic (20–22). Several syndromes, however, will be discussed due to their propensity to result in death.

Respiratory

Infections of the lower and upper respiratory system comprise a significant proportion of both community-acquired and nosocomial infections. As mentioned previously, influenza and pneumonia are among the leading causes of total deaths in the U.S. Many of the agents of community-acquired pneumonia are easily identifiable if antemortem cultures or rapid diagnostic tests were performed. These results may be supplemented with postmortem pulmonary cultures, and the results from both should be correlated with histopathologic findings. At times, cultures will not be available because death was sudden, infection was not suspected, infection was due to a fastidious pathogen, or prior antibiotic therapy affected culture results. In these instances, direct detection of the pathogen in tissue, using immunohistochemistry (IHC) or in situ hybridization techniques (ISH), should be attempted. These techniques also have the advantage of demonstrating the organism and its associated tissue response.

Central Nervous System

Central nervous system (CNS) infections may present acutely (for example, meningococcal meningitis), subacutely (enteroviral meningitis), or chronically (cryptococcus meningitis). Knowing the tempo of the disease is key to narrowing the differential diagnosis. In bacterial meningitis, although onset is rapid, antecedent respiratory symptoms are common. Although most cases of bacterial meningitis are due to hematogenous spread, search for a contiguous focus of disease (otitis media, sinusitis) is important in deciphering the pathogenesis of disease. Many infections have distinct seasonal and geographic predilections, which further narrows the differential diagnosis. Cerebrospinal fluid (CSF), meninges, and CNS parenchyma may be obtained for bacterial, viral, and fungal cultures. Certain organisms such as rickettsia cannot be cultured but may show histological changes that can be evaluated further with IHC or molecular methods. Molecular techniques are extremely useful in deciphering CNS infections (23–25), particularly those due to viruses (26,27).

When an autopsy is performed for a neurodegenerative disease, special protective measures are necessary as discussed below in the section of personal protection.

Septicemia

In patients with no antecedent history or underlying conditions, septic shock is often due to aerobic gram-negative bacilli or gram-positive cocci. The list of potential pathogens, however, expands if the patient is neutropenic, asplenic, or suffers from other predisposing conditions,

including congenital or acquired immunodeficiency states. Although bacteremic sepsis is most common, sepsis may complicate infections with viruses, rickettsia, fungi, mycobacteria, and parasites. Approaching these cases with a broad differential diagnosis and purposeful tissue sampling is important in delineating the causative agent.

Fever and Rash

The acutely ill patient with fever and rash connotes a broad differential diagnosis, which can be narrowed somewhat with a thorough consideration of the characteristics of the rash (28). Although many infections may present with fever and rash, those most likely to lead abruptly to death include meningococcemia, rickettsial infections, and toxic shock syndrome. When evaluating the rash, it is important to note 1) characteristics of the lesions, 2) distribution of the rash, 3) presence of petechiae, and 4) timing and progression of the rash relative to fever and death. Meningococcemia characteristically produces petechial lesions on the trunk, extremities, and mucous membranes. Rocky Mountain spotted fever, a prototypical rickettsial infection, starts as a maculopapular rash that spreads centripetally and evolves eventually to petechiae. Toxic shock syndrome (TSS) produces a sunburn-type rash that desquamates usually after 1–2 weeks. Whereas blood cultures may yield the causative agent in meningococcemia, cultures would be negative in rickettsial infections. A skin biopsy, however, may allow the pathologist to identify the organisms using immunohistochemical stains of formal-fixed, paraffin-embedded tissue. In cases of suspected TSS, vaginal and wound cultures may be useful in elucidating the reservoir of bacteria. In a report on the autopsy of a 15-year-old girl with tampon-related TSS, Blair and colleagues demonstrated extensive superficial ulcerations and thrombophlebitis of the vagina, with bacteria on the mucosal surface only (29).

Cardiovascular Infection

Although cardiovascular manifestations often complicate sepsis, cardiovascular infections that may culminate in unexplained death include acute infective endocarditis and myocarditis. A recent case report in the *New England Journal of Medicine* illustrates the rapid deterioration that accompanies invasive endocarditis (30). In this case, infection spread from the infected valve into adjacent cardiac tissue producing acute pulmonary edema and progressive conduction abnormalities. This case, which was possibly due to bartonella, also illustrates the role of serologic tests in cases where premortem cultures are negative.

Cases of fulminant, acute infective endocarditis (IE) are more likely to be caused by virulent organisms such as *Staphylococcus aureus* or *Streptococcus pneumoniae* (31). Autopsy case reports and case series have provided useful information regarding the pathogenesis of suspected IE, IE in sudden unexpected deaths, and IE due to unusual pathogens (32–37).

There are multiple infectious etiologies of myocarditis, of which viruses, particularly group B coxsackieviruses, predominate in the U.S. When acute, myocarditis may present with cardiogenic shock, myocardial infarction, or lethal arrhythmias. In a study of sudden death in young military recruits in the U.S., the major cause of sudden death was an identifiable cardiac abnormality (64 of 126 recruits, 51%), including 13 cases of myocarditis (38). In a study of fatal myocarditis in Italy, the authors concluded that myocarditis is often underdiagnosed antemortem. In the Italian study of 2,600 autopsies, 143 cases of active myocarditis were diagnosed, and myocarditis was ruled as the cause of death for 39; only one case was suspected antemortem (39). These studies highlight the need for a high index of suspicion for myocarditis and consideration for obtaining cardiac tissue for viral culture, immunohistochemical analysis, and/or PCR.

Skin and Soft Tissue Infections

Skin and soft tissue infection, including necrotizing fasciitis and myositis, may follow a trivial injury or may show no obvious portal of entry. These infections proceed rapidly and may present to the medical examiner (ME) as an obscure autopsy (40–43). When due to a single pathogen, the usual culprits are group A streptococcus, *S. aureus*, and anaerobic streptococci. Polymicrobial infections contain a mixture of aerobic and anaerobic bacteria, sometimes numbering up to 15 different organisms. According to Gorbach, polymicrobial infections should be considered in four settings: 1) following surgical procedures, 2) infection proceeding from a decubitus ulcer or perianal abscess, 3) in intravenous drug users, and 4) spread from a Bartholin's abscess or minor vulvovaginal infection (44). The premortem diagnosis of a deep skin and soft tissue relies on a constellation of findings that include bullous lesions, gas in the soft tissues, and devitalization of fascial planes, all of which may be confused with postmortem degradation or decomposition. A high index of suspicion for the presence of a necrotizing soft tissue infection must be maintained. Appropriate cultures and full-thickness biopsies should be taken.

Gastrointestinal

Gastrointestinal complaints are frequent nonspecific symptoms that accompany a variety of syndromes. When the gastrointestinal tract is the primary site of disease, those conditions most likely to produce death include bacillary dysentery, enteric fever, and amebic colitis/hepatic abscess (45). *Shigella dysenteriae* is the most virulent Shigella, producing classic dysentery that may be complicated by colonic ulceration, perforation, and peritonitis. At autopsy, there is

extensive colonic ulceration with patchy to nearly continuous involvement. According to Kelly and Owen, most fatal cases show involvement of the entire colon and terminal ileum (46). Typhoid (enteric) fever is a severe systemic infection, the complications of which include pneumonia, hepatitis, myocarditis, meningitis, orchitis, and osteomyelitis. Perforation, hemorrhage, and sepsis are associated with high case fatality rates. Cultures of blood, stool, and urine are positive late in infection and remain the gold standard for antemortem diagnosis. As with other pathogens, molecular techniques are evolving to allow for a more rapid diagnosis and may be useful in the postmortem analysis (47–49).

Liver

Hepatic failure may be a manifestation of sepsis and multiorgan failure or may be the isolated manifestation of infectious hepatitis, a consequence of infection by any of the hepatotropic viruses, hepatitis A, B, C, D, and E. Fulminant hepatitis is defined as severe hepatitis, which leads to liver failure and hepatic coma within 8 weeks (50). Without liver transplant, most patients with fulminant hepatic failure die. Although there are histopathologic clues to the etiologic agent, definitive diagnosis relies upon serologic data and immunohistochemical analysis (51). In addition to the usual hepatotropic viruses, fulminant hepatic failure may result from infection with adenovirus, varicella, bacteria, and fungi (52–56).

Detection of Infectious Agents

After careful review of the patient's antemortem history, including positive and negative cultures and pertinent serum studies, it is time to proceed with the necropsy. In order to protect oneself from contracting or spreading an infectious agent, proper personal protective equipment (PPE) must be utilized and is discussed in a subsequent section. In addition to PPE the autopsy suite should have blood collection vials with and without EDTA, sterile specimen containers, blood culture bottles and culture swabs readily available and marked with the patient's name and unique identifier.

Before dissection, a thorough external examination must be performed. This includes close inspection of any skin abnormality, conjunctivae of the eyes, oral and nasal mucosa, auditory canal, genitourinary orifices and the rectum. If clinical suspicion for an infectious agent involving these areas exists, it may be necessary to culture one or more of the areas using a swab technique or take a section of tissue, such as a punch biopsy of skin, for further histological analysis.

In a review of autopsy microbiology, Reznicek and Koontz discuss the role of standard microbiologic cultures in the performance of an autopsy (2). The number, site, and type of cultures taken is dependent upon antemortem history, gross appearance of the organs, and immune status of the patient. Even if all organs are grossly unremarkable, cultures of blood and spleen should be taken. If the patient was immunocompromised, viral, mycobacterial, and fungal cultures may be needed in addition to standard bacteriology cultures. They offer the following recommendations for obtaining cultures at necropsy: 1) after making the initial incision, visually inspect the cavities for fluid collection and culture them before additional manipulation and culture organs that are clinically suspected of harboring infection; 2) dry the area to be cultured by searing with a hot steel spatula; 3) for tissue cultures, remove 1-cm^3 portion of tissue with sterile instruments and place in a properly labeled, sterile Petri dish; 4) aspirate blood from the right atrium for aerobic and anaerobic blood cultures; 5) aspirate urine directly from the bladder and place in sterile tube; 6) obtain CSF from the lateral ventricle after the skull cap is removed or by cisternal tap through skin; 7) culture abscesses and granulomata from the center and periphery of the lesion; 8) use different sets of sterile instruments for each culture/site; and 9) divide the specimens aseptically for the various laboratories and deliver promptly.

An alternative method for obtaining blood cultures is the iodine-subclavian technique, which has been shown to reduce the false-positive rate of blood cultures as compared to the traditional technique of atrial heat searing (57). Using a similar technique, cerebral spinal fluid can be obtained using a posterior approach.

During necropsy, special attention should be given to the evaluation of lymph nodes and the organ systems involved in the antemortem clinical syndrome, if any. Because an infectious etiology is not always suspected before and even during autopsy, representative sections from each organ must be formalin fixed for further microscopic evaluation. If an infectious agent is suspected, sections from presumptive infected organs, most often the lungs, should be submitted for culture in sterile containers using the searing method and sterile instruments along with swab cultures. Snap-freezing tissue with isopentane and liquid nitrogen and fixing tissue with glutaraldehyde should also be considered. Once the necropsy is finished, it is impossible to go back and obtain more tissue or cultures.

Although cultures are often helpful in isolating an infectious agent, they often produce contaminants and results may be difficult to interpret (58).

An important part of the autopsy interpretation is correlating the clinical, histopathologic, and microbiologic findings. Discerning whether the cultured organism is a true pathogen or a contaminant can be difficult. Reznicek and Koontz offer several points for consideration when interpreting culture data: 1) Could the organism be a contaminant? 2) Did spleen and blood cultures grow the same organisms, supporting pathogenesis? 3) Was the same organism cultured from multiple sites? 4) Is the clinical course compatible with sepsis? 5) Are organisms present on tissue sections? 6) Were organisms present on gram stains of tissue homogenates?

7) Is there a tissue host response compatible with the putative infection? 8) Do autopsy findings correlate with antemortem clinical diagnoses?

In addition to the technical problems associated with autopsy cultures, many organisms and emerging infectious agents are not detected by this methodology. Thus, a system incorporating histological and molecular techniques must be utilized.

The initial step in identifying a pathologic infectious agent is through examination of formalin-fixed, paraffin-embedded tissue that has been stained with hematoxylin and eosin (H&E). These tissue sections provide information about the overall tissue reaction, if any, to the offending agent. In addition, viral inclusion bodies and some bacteria and fungi can be detected and identified on H&E stained slides. Using this information, a differential diagnosis for the reaction pattern can be constructed.

The next step in identifying the infectious agent is through the use of special stains. According to Chandler (59), one of the most important special stains is the silver impregnation for bacteria. Silver impregnation stains, such as the Warthin–Starry, blacken all bacteria non-selectively including non-gram-reactive bacteria (e.g., *Treponema pallidum, Borrelia burgdorferi, Leptospira* spp. and *Bartonella* spp.) and small weakly gram-negative bacilli (e.g., *Legionella* spp. and *Francisella tularensis*). Although the black coating of the silver stain may distort the morphology of the bacteria, silver impregnation procedures are more sensitive than tissue gram stains especially when only a small number of bacteria are present. After bacteria have been detected, tissue gram stains can be utilized to distinguish between gram-positive and gram-negative bacteria. The Brown and Brenn procedure is superior for demonstrating gram-positive bacteria and the Brown–Hopps procedure is superior for demonstrating gram-negative bacteria and rickettsiae.

Another equally important special stain is the Gomori's methenamine silver (GMS) stain. This is the stain of choice for identifying fungi. In addition to blackening degenerated and fragmented fungal organisms, this method also blackens a whole host of non-fungal pathogens. For example, actinomycetes, nocardiosis, *Mycobacterium* spp., *Streptococcus pneumoniae, Klebsiella pneumoniae, Haemophilus influenzae,* the cyst walls of *P. carinii* and the cytoplasmic granular inclusion bodies of cytomegalovirus all stain with this method.

Conventional acid-fast stains, such as Ziehl–Neelsen stain, highlight *Mycobacterium* spp. However, if the number of organisms is low, the auramine–rhodamine fluorescence procedure has greater sensitivity. Modified acid-fast stains such as the Fite and Kinyoun stains will highlight *Nocardia* spp., some forms of *Legionella* spp. and *Mycobacterium leprae*.

Other miscellaneous stains that may be helpful include mucicarmine for the identification of *Cryptococcus neoformans*; Giemsa for the identification of protozoans, rickettsiae and chlamydiae; melanin for identification of dematiaceous fungi, and connective tissue stains for the identification of *Trypanosoma cruzi* and the morphology of nematodes, cestodes and trematodes.

Although these classical stains are extremely useful, many organisms are unapparent on morphologic examination or have ambiguous characteristics that are not specific to one organism. In these situations, IHC techniques or molecular biologic techniques such as PCR may provide definitive identification. Both of these techniques can be performed on formalin-fixed, paraffin-embedded tissue.

IHC combines immunologic and histochemical techniques for the detection of phenotypic markers on organisms. The central theme in this technique is the binding of an antibody to a corresponding antigen. Immunoenzymatic or immunofluorescence methods are then utilized to visualize the binding of these complexes. This procedure can be utilized on formalin-fixed, paraffin-embedded tissue. In order to enhance the immunolabeling of viral antigens, tissue sections are often predigested with a proteolytic enzyme in order to free cross-linked antigens. This predigestion step is not usually necessary for bacteria, fungi and protozoans since their antigenicity is not appreciably affected by the fixation and embedding. Thus, IHC can be very helpful in the diagnosis and confirmation of viral diseases, some bacteria, fungi, protozoa and parasites. Many monoclonal antibodies and polyclonal antisera are commercially available. Table 1 lists commercially available antibodies for various infectious agents (60). The asterisk designates those infectious agents that have been identified by the CDC as potential biologic terrorism agents. In addition, the CDC also has

Table 1. List of infectious agents that can be identified on formalin-fixed, paraffin-embedded tissue using commercially available antibodies and/or antisera

Virus	Bacteria	Fungi, protozoa and parasites
Adenovirus	*Borrelia burgdorferi*	*Aspergillus* sp.
Coronavirus	*Campylobacter* sp.	*Blastomyces* sp.
Cytomegalovirus	*Escherichia coli**	*Candida* sp.
Hantavirus*	*Helicobacter pylori*	*Cryptococcus neoformans*
Hepatitis A	*Klebsiella*	*Cryptosporidium* sp.*
Hepatitis B	*Legionella*	*Entamoeba histolytica*
Hepatitis C	*Mycobacterium**	*Fusarium anthophilum*
Herpes Simplex Virus	*Pseudomonas aeruginosa*	*Giardia lamblia*
		Histoplasma capsulatum
HIV-1	*Salmonella**	*Leishmania* sp.
HIV-2	*Shigella**	*Pneumocystis carinii*
JC virus	*Staphylococcus**	*Sporothrix schenckii*
Parvovirus	*Streptococcus*	*Toxoplasma gondii*
Picornavirus	*Treponema pallidum*	*Trichomonas* sp.
RSV	*Yersinia**	*Trypanosoma cruzi*
Varicella-Zoster virus		

*Infectious agents that have been designated as potential biologic terrorism agents by the CDC.

antibodies and antisera to detect *Bacillus anthracis, Francisella tularensis* and variola virus (61).

Another diagnostic tool, PCR, has proven invaluable in the identification of emerging infections, especially those caused by viruses. The main purpose of PCR is to amplify targeted nucleic acids, either microbial DNA or RNA sequences. Traditional PCR amplifies DNA sequences and a technique known as reverse transcription PCR (RT-PCR) can be utilized to identify viral RNA sequences. These methods can detect any infectious agent for which even limited DNA and RNA sequence information is know. Although fresh or frozen tissue is preferred, these methods can be performed on fixed, paraffin-embedded tissue. Another advantage of the method is that many samples can be done simultaneously.

One of the major disadvantages of traditional PCR is that one cannot directly observe the defined nucleic acid sequences in tissue sections. One method that allows for this visualization is ISH. This procedure, however, is technically demanding and its sensitivity is comparable to that of IHC (60). An emerging technologic advance that will allow for the direct visualization of the nucleic acid sequences in intact tissue sections is in situ PCR. This method employs the use of RT-PCR directly on slide-mounted tissue sections (62).

Historically, electron microscopy has been utilized for the identification of emerging infectious agents. For example, electron microscopy was vital to the identification of *Legionella pneumophila* in the late 1970s (63). Electron microscopy has the advantage of not relying on presumptive etiologic agents as is necessary with the previous techniques. The main disadvantages are that the tissue must be fixed in glutaraldehyde and the presumptive organism must be present in the small sample of tissue that is submitted.

During the 1990s, the techniques described above in addition to close attention to clinical syndrome and patient's history were vital in discovering the causative agents of two emerging infectious diseases: hantavirus pulmonary syndrome and West Nile encephalitis. In the case of hantavirus, IHC method using antiserum to hantaviral nucleocapsid antigen was critical in the early understanding of the hantavirus pulmonary syndrome (64). In the case of West Nile virus, histologic examination of the central nervous system revealed necrosis in the gray matter with infiltrates of microglia and polymorphonuclear leukocytes. These sections were then stained using IHC techniques that utilized an antibody to St. Louis encephalitis virus (SLE), a member of the *Flavivirus* family. Confirmatory tests using PCR were negative when SLE virus-specific primers were used. In addition, animals not usually associated with SLE outbreaks, such as crows and other birds, were reported to have increased fatalities. One explanation of these differing results was that the antibody used in IHC was known to cross-react with several other members of the *Flavivirus* serocomplex including SLE, Japanese encephalitis virus and West Nile virus. RT-PCR techniques were then performed utilizing several degenerate primer sets designed to detect a wide variety of flaviviruses. These tests concluded that West Nile virus was the causative agent (65).

These two outbreaks underscore the need to incorporate clinical syndrome, traditional histologic techniques, and advance diagnostic methods in the detection of infectious agents.

Infectious Disease and Autopsy Personnel

It has been well documented in the scientific literature that autopsy room personnel are at risk for acquiring infectious diseases from the deceased. In fact, it has been stated that the Father of Histology, Xavier Bichat (1771–1802), succumbed to *Mycobacterium tuberculosis* acquired while doing some 600 autopsies in the year of his death (66). In order for an infectious agent to be transmitted from a patient to autopsy room personnel and cause disease, three things are necessary. First, the deceased patient must harbor an infectious agent that has remained viable after death. Second, there must be a route of transmission. This may be in the form of aerosolized agents, direct skin/mucosa contact with an infectious agent or accidental inoculation of an infectious agent. Third, the host/autopsy room personnel must be susceptible to the infectious agent and manifest disease.

The type of infectious agent the patient has, or is suspected of having, plays a significant role in the handling of the autopsy. The Centers for Disease Control and Prevention (CDC) classifies the handling of infectious disease cases according to biosafety levels (BSLs) ranging from 1 to 4, with BSL-1 encompassing agents not known to consistently cause disease in healthy adults to BSL-4 encompassing dangerous or exotic agents that pose a high risk of life-threatening disease and related agents with unknown risk of transmission (see Table 2). Autopsies on patients with infectious agents that fall into the BSL-4 level must be done in facilities adequate to handle them. Most common diseases, however, fall into category BSL-3 or lower. It cannot be overemphasized that the infectious agent is not always known or suspected during the time of necropsy.

The route of transmission differs for different infectious agents. For example, *Mycobacterium tuberculosis* can be

Table 2. Biosafety levels

BSL-1	Infectious agents not known to consistently cause disease in healthy adults
BSL-2	Infectious agents known to cause human disease via auto-inoculation, ingestion and mucous membrane exposure
BSL-3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences
BSL-4	Dangerous or exotic agents that pose a high risk of life-threatening disease and related agents with unknown risk of transmission

transmitted either through direct contact with open wounds or through aerosolized airborne particles. In contrast, human immunodeficiency virus (HIV) is blood borne and transmitted either by mucosa, open wound or direct inoculation.

In the clinical setting, these differing routes of transmission have led to the implementation of three different types of precautions: airborne, droplet and contact. Airborne precautions are utilized for infectious agents that remain suspended in the air in the form of droplet nuclei and are transmitted if inhaled. Droplet precautions are utilized for infectious agents that are transmitted by large droplets traveling up to 6 feet and are no longer transmissible when they fall to the ground. Contact precautions are utilized for infectious agents that can be transmitted from environmental surfaces and equipment.

Unlike surgeons who wear personal protective equipment (PPE) primarily to reduce the risk of transmission of infectious agents from provider and environment to patient, autopsy personnel wear PPE to reduce the transmission of infectious agents from deceased to provider. This PPE should protect against fluids coming in contact with the skin and mucosa as well as to reduce or prevent the breathing of aerosolized agents generated during the autopsy.

The minimum recommended PPE include surgical scrubs, surgical cap, impervious gown and/or apron with full sleeve coverage, eye protection (face shield recommended), face mask, shoe covers or waterproof shoes and double surgical gloves. If available, the gloves should be interposed with a cut-proof synthetic mesh or Kevlar. These gloves should be changed frequently. It must be noted that a normal surgical mask will not protect against certain infectious agents, and it is the authors recommendation that a N-95 or higher mask be worn at all times.

The susceptibility of the autopsy room personnel to disease depends on the immunologic state of the personnel. This state can be influenced with the use of vaccines such as the hepatitis B vaccine. It is not recommended that immunocompromised personnel or personnel with open wounds take part in autopsies.

Potentially Hazardous Infectious Agents Encountered at Autopsy

This section discusses some of the classic and emerging infectious agents that can cause significant morbidity and mortality to the autopsy personnel. For each infectious agent, the mode of transmission, risk of transmission and measures to avoid transmission are discussed.

Mycobacterium tuberculosis

Mycobacterium tuberculosis is the acid-fast bacillus that is responsible for pulmonary tuberculosis. Although there are historical cases of cutaneous manifestations of *Mycobacterium tuberculosis* transmitted either by direct inoculation or the non-use of gloves, the majority of cases in autopsy personnel are pulmonary in nature. In the case of pulmonary tuberculosis the mode of transmission is through inhalation of aerosols containing tubercle bacilli.

The literature supports the theory that even brief exposure at autopsy carries a high risk of transmission and subsequent infection. Although often suspected and diagnosed before death, pulmonary tuberculosis can go unrecognized until after the autopsy is completed. Although exact transmission rates have not been described in the literature, Templeton et al. discuss a case of a patient who died of unsuspected tuberculosis in which all five Mantoux-negative people present at autopsy converted from negative to positive as compared to 40 Mantoux-negative health care workers who had direct patient care with the patient but remained negative. In addition, two of the people present at the autopsy developed positive sputum cultures (67). This highlights the fact the precautions must be taken during all autopsies.

The CDC recommends the use of an N-95 or N-100 mask in addition to the standard PPE. In addition, they recommend that all autopsy personnel have the Mantoux test conducted yearly.

Human Immunodeficiency Virus (HIV)

The human immunodeficiency virus is a RNA retrovirus. This is the virus that is responsible for acquired immunodeficiency syndrome (AIDS). HIV can be transmitted via percutaneous or mucocutaneous inoculation. Aerosol transmission rate has not been documented. Percutaneous inoculation can occur when the deceased's blood comes in contact with uncovered wounds or after a needle stick, scalpel injury or injury from a sharp object such as bone. Mucocutaneous inoculation occurs when infected blood is splashed onto the mucous membranes.

The risk of seroconversion depends on many factors including viral load, amount of blood inoculated and susceptibility of the autopsy personnel (i.e., immunosuppressed or receiving post-exposure prophylaxis). Factors known to increase the risk of seroconversion in health care workers include deep injury, terminal illness of patient (high viral load) and visible blood on the device (68). Importantly, these factors are often the ones encountered at autopsy with an accidental scalpel injury. The estimated HIV transmission rate after a single percutaneous inoculation ranges from 0.10 to 0.36%. The estimated risk after mucocutaneous exposure ranges from 0.04 to 0.63% (68).

Although HIV does not survive well outside of the body, numerous studies have shown that HIV can be isolated from various tissues, including cranial bone, cerebrospinal fluid, spleen and blood 5 days after death despite being stored at 6°C. Standard PPE and careful dissection techniques help further reduce the risk of transmission.

Hepatitis B and C

Hepatitis B virus is a double-stranded DNA virus and hepatitis C is a single-stranded, positive-sense RNA virus. Like HIV, the route of transmission of both of these viruses can be either percutaneous or mucocutaneous. Although HBV is highly infective, ten times more infective than HBC, occupational transmission has decreased dramatically due to the wide use of vaccination programs. In contrast, HCV has been reported to be less infectious. However, no vaccine exists and the risk of transmission in health care workers after percutaneous exposure is 2.7–10% (69). Thus, it is important to use standard PPE and careful dissection technique.

Severe Acute Respiratory Syndrome (SARS)

SARS is a recently defined viral respiratory illness that is caused by a coronavirus that has been designated SARS-associated coronavirus. This disease was first reported in Asia in 2003 and within months spread over four continents infecting over 8,000 people and killing almost 800 (70). Although the exact mode of transmission of the virus is not known, experts believe that the virus is transmitted most readily by respiratory droplets from an infected individual who comes in contact with the mucous membranes of another person. This can occur either by direct spread through the air or indirectly from touching objects contaminated with infective respiratory droplets.

Although the risk of transmission to autopsy personnel is not currently known, the CDC recommends that, in addition to standard autopsy PPE, all personnel wear additional respiratory protection (N-95 or N-100 disposable particulate respirators) if aerosols are generated (71). Ideally, autopsies should be done in autopsy suites with adequate air-handling systems that have downward exhausts that are either direct to the outside or passed through a HEPA filter if the air is re-circulated. Biosafety cabinets should be used for the handling and examination of smaller specimens, and vacuum shrouds used for oscillating saws to contain aerosols and reduce the volume of aerosol released in the autopsy suite.

Prions

Prions are proteinaceous infectious particles that lack nucleic acids. This agent is responsible for neurodegenerative diseases of humans that have been referred to as the transmissible spongiform encephalopathies (TSEs). Known TSEs include Kuru and Creutzfeldt-Jakob disease (CJD).

Prion disease is thought not to be communicable or contagious during life. There is no documentation of the transmission of prions via blood droplets, cerebrospinal fluid or exposure to intact skin or mucous membranes. However, a theoretical risk exists. During autopsy, BSL-2 precautions should be used. The CDC recommends that the entire brain be collected at autopsy while the head is in a plastic bag to reduce aerosolization and placed in a plastic bag for weighing. It should then be cut in coronal section, heat sealed in a heavy-duty plastic bag and stored at −70°C.

Although there is no documentation on the transmission of prion disease at autopsy, no effective treatment exists and caution must be taken. Animal studies have shown that there are high concentrations of prions in the central nervous system and its coverings as well as in the spleen, lymph nodes and lungs. Thus, it is recommended that only the brain be taken if possible and that respiratory devices be utilized in addition to standard PPE (72).

Table 3. Categorization of potential bioterrorism agents/diseases

Category A (definition below)
Anthrax (*Bacillus anthracis*)
Botulism (*Clostridium botulinum* toxin)
Plague (*Yersinia pestis*)
Smallpox (*Variola major*)
Tularemia (*Francisella tularensis*)
Viral hemorrhagic fevers
Category B (definition below)
Brucellosis (*Brucella* sp.)
Epsilon toxin of *Clostridium perfringens*
Food safety threats (e.g., *Salmonella* sp., *Shigella*)
Glanders (*Burkholderia mallei*)
Melioidosis (*Burkholderia pseudomallei*)
Psittacosis (*Chlamydia psittaci*)
Q fever (*Coxiella burnetii*)
Ricin toxin from *Ricinus communis* (castor beans)
Staphylococcal enterotoxin B
Typhus fever (*Rickettsia prowazekii*)
Viral encephalitis
Water safety threats (e.g., *Vibrio cholerae*, *Cryptosporidium*)
Category C (definition below)
Emerging infectious diseases such as Nipah virus and Hantavirus
Category A Diseases/Agents: High priority agents include organisms that pose a risk to national security because they can:
Easily be disseminated or transmitted from person to person
Result in high mortality rates and have the potential for major public health impact
Possibly cause public panic and social disruption
Require special action for public health preparedness
Category B Diseases/Agents: Second highest priority agents include those that:
Are moderately easy to disseminate
Result in moderate morbidity rates and low mortality rates
Require specific enhancements of CDC's diagnostic capacity and enhanced disease surveillance
Category C Diseases/Agents: Third highest priority agents include emerging pathogens that could be engineered for mass dissemination in the future because of:
Availability
Ease of production and dissemination
Potential for high morbidity and mortality rates and major health impact

Role of Autopsy in Suspected Bioterrorism

In addition to the importance of the autopsy in evaluating cases of naturally occurring deaths, pathologists have an

important role in recognizing deaths due to intentional release of a biologic agent. Public health bulletins and educational courses have been provided to the medical community at large regarding recognition of illness associated with bioterrorism.

Certain epidemiologic clues may alert health care personnel to a potential bioterrorism event, including several that are germane to autopsy personnel: 1) an endemic disease emerging at an uncharacteristic time or in an unusual pattern, 2) clusters of patients from a single locale, 3) large numbers of rapidly fatal cases, and 4) disease that is uncommon and has bioterrorism potential.

The CDC has categorized potential bioterrorism agents into three categories based upon potential to be used as a bioterrorism weapon, ease of dissemination and transmission, and potential for major public health impact (Table 3). Given the high case fatality rates associated with the diseases in Category A, pathologists, particularly MEs, will be involved in deciphering these cases. Autopsies performed for potential investigation of potential bioterrorism should accomplish three goals: 1) establish the disease process and the etiologic agent, 2) determine that the agent of disease is the cause of death, and 3) rule out competing causes of death (61). A partial list of diagnostic specimens and testing is given in Table 4. A thorough guidebook for MEs and coroners is available through the CDC website (www.cdc.gov).

To accomplish the goals addressed above, the ME should be able to 1) perform complete autopsies with histologic sampling of multiple organs; 2) have access to routine microbiologic testing for organism-specific diagnoses in potential infectious disease deaths; 3) ensure protection from both airborne and bloodborne pathogens for all occupants of the autopsy facility; 4) participate in standardized surveillance for infectious disease mortality; and 5) document death investigative information on standard searchable forms that can be shared with public health authorities (61). Importantly, these goals should be accomplished while maintaining chain of custody for specimens at all times.

In the U.S., the CDC has collaborated with other agencies to establish the Laboratory Response Network linking local, state, and federal laboratories, each of which has been assigned a designation based upon diagnostic capability. It is critical that autopsy personnel be familiar with where their initial specimen submissions should go, as that lab will be their point of contact for test results. Except for specimens potentially containing smallpox, all specimens to be tested for potential biological terrorism agents are handled in this manner. Testing for smallpox is done in a biosafety level IV facility, such as the CDC or the U.S. Army Medical Research Institute of Infectious Diseases (USAMRID). MEs and other pathologists performing autopsies should establish close working relationships with local health departments to facilitate two-way communication regarding potential infectious disease cases.

Table 4. Recommended diagnostic specimens and tests for selected biological agents

Agent	Syndrome	Diagnostic specimens	Diagnostic tests*
Bacillus anthracis	Cutaneous Gastrointestinal Inhalational	Skin at center and periphery of eschar; pleural fluid cell block, pleural tissue, mediastinal lymph node	H&E Gram Silver stain IHC
Yersinia pestis	Bubonic Pneumonic Septicemic	Histologic sampling of multiple organs; hemorrhagic lymph nodes; lungs	H&E Gram Silver stain Giemsa IHC DFA
Francisella tularensis	Ulceroglandular Oculoglandular Glandular Pharyngeal Typhoidal Pneumonia	Histologic sampling of multiple organs; necrotic lymph nodes should be sampled; culture potential portal of entry (skin, throat, conjunctiva)	IHC DFA of formalin-fixed tissue
Clostridium botulinum toxin	Descending paralysis	Tissue for anaerobic cultures from suspected entry sites (wound, GI, respiratory) intestinal contents	Microbiologic culture Botulinum toxin mouse bioassay
Variola virus	Smallpox	Fluid from vesicle for EM	EM IHC
Multiple viruses, including Ebola, Marburg, Flaviridae	Viral hemorrhagic fevers	Histologic sampling of multiple organs; serum; skin samples	PCR IH EM

*Data from MMWR 2004 (61).

In summary, the modern autopsy continues to be a vital tool for determining an individual's cause of death. It also remains an important tool for furthering our understanding of disease pathogenesis, and for identifying potential disease outbreaks, either natural or malevolent.

Disclaimer: The opinions presented in this paper are those of the authors and do not represent the opinion of the U.S. Air Force or the Department of Defense.

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