Applied Biosafety, 6(1) pp. 19-26 © ABSA 2001

Risk Assessment for Working with Infectious Agents in the Biological Laboratory

Richard C. Knudsen

Centers for Disease Control and Prevention, Atlanta, Georgia

Abstract

Seventeen risk factors for working with infectious agents in the laboratory and animal facility environment are identified and discussed. The risk factors are useful in performing qualitative risk assessments to determine the biosafety level necessary to work safely with an infectious agent or to initiate modifications to the practices and procedures, equipment and facility requirements at each biosafety level.

Introduction

Workers in biological laboratories are potentially exposed to the risks, or hazards, of infectious agents and their toxins which can cause them serious harm or death (Collins, 1983; Sewell, 1995; Sulkin & Pike, 1949, 1951; Sullivan et al., 1978). To work with these biohazardous agents, the risk of exposure to them during the work process must be assessed. This process is called risk assessment and requires the identification of the various risk factors. Each risk factor must be evaluated and then processes and controls implemented which will minimize, to the lowest possible degree, exposure to the biological agents. This latter process is risk management. Risk assessment and risk management go hand-in-hand and may be viewed as the two sides of the same coin.

Assessing the Biosafety Levels

The risk assessment process for working with infectious agents in laboratory or animal facilities is aimed at reducing to a minimum the risk of working with these agents by placing the agent in any one of four biosafety levels (BSLs), termed BSL 1, 2, 3, and 4, as defined by the CDC/NIH guidelines *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) (Richmond & McKinney, 1999). Each biosafety level has specific facility, equipment, and practice requirements for working with the assigned infectious agents. The risk assessment process is also used for enhancing or modifying facility, equipment, and usually the practices and procedures at each biosafety level. Some examples include the use of BSL-3 practices with BSL-2 facilities, the use of high efficiency performance (HEPA) filters on BSL-3 laboratory air exhaust systems, and the addition of waste treatment and shower out facilities to BSL-3 laboratories when working with USDA-restricted animal and poultry pathogens. A complete discussion of the four biosafety levels is found in the BMBL.

BSL-1 is appropriate for work with defined and characterized strains of microorganisms that are not known to cause disease in healthy adult humans. BSL-2 is appropriate for a broad spectrum of indigenous moderate-risk agents present in the community and associated with human disease of varying severity. BSL-3 is appropriate for work with indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious and potentially lethal infection. BSL-4 is appropriate for dangerous and exotic agents which pose a high individual risk of life-threatening disease which may be transmitted by the aerosol route and for which there is no available vaccine or therapy.

Qualitative vs. Quantitative Risk Assessment

Risk assessments can be either quantitative or qualitative. The most effective risk assessments are quantitative, or measurable, and are often used for those chemical hazards or radiological hazards which can be readily measured. However, biological agents are not uniform chemical moieties for which well developed and relatively easy-to-perform assays are available. They are a complex of thousands of interreactive biochemical macro and micro molecules such as proteins, lipids, carbohydrates, nucleic acids and their building blocks united into discrete units which have the capacity to reproduce themselves in the host's cells, tissues, and body fluids. There is also an incredible variety of infectious agents, ranging from bacteria, rickettsia, viruses, yeasts, molds, uni- and multicellular parasites, and prions. Each species of agent may have subtypes, strains, and variants that differ from the parent in virulence, host range, transmission, sensitivity to antimicrobial agents, etc. Because of their chemical complexity and biological diversity, no single chemical test can be used to identify a particular species of microorganism much less its related variants and subtypes.

The most defining characteristic of an infectious agent is its ability to replicate in the host (Knudsen, 1999). Viruses and rickettsia can only replicate in living cells. Bacteria, yeasts, and molds can replicate in extracellular spaces and body fluids. Damage to the host is not caused by the initial invading microbes but by their descendants, replicated many thousands or millions of times over in the host and oftentimes spreading to other organs and tissues for further replication. Thus, the most quantitative measure of an infectious agent is its ability to replicate. However, assays of replicability are difficult to perform, time-consuming, costly, and can be used only for a limited number of agents. Because such assays are usually done *in vitro* or in surrogate cells or animal hosts, they are also subject to considerable interpretation when trying to extrapolate these results to the human host.

It is the biological diversity of microorganisms, their complex chemical structure, their multiple methods of transmission, their complex interactions with the host, and their unique requirement to replicate within the host that make it difficult to define meaningful assays that might be used to identify them in quantitative assays. Because quantitative risk assessments cannot usually be adequately performed, it is necessary to rely on qualitative risk assessments.

Qualitative Risk Assessment: Risk Factors

The following paragraphs discuss the major risk factors that are normally encountered in working with infectious agents in the laboratory environment. Some of these risk factors, such as disease severity, transmission mode, and availability of prophylactic measures, are critically important in determining the biosafety level. Other risk factors are important for suggesting areas where additional protective measures are needed within the biosafety level.

Agent Identity and Characterization

The identity of an agent is the key to the information-gathering process. If the identity of the agent is known or strongly suspected, it can be determined if it is a well characterized or a poorly characterized agent. If the agent is well characterized published disease information (Benenson, 1995), agent summary statements, and recommended biosafety levels (or risk groups) will be available (Kennedy, 1996; Richmond & McKinney, 1999; USDHHS, 1994). The history of laboratory-acquired infection with the agent may be well documented in the literature and review articles (Collins, 1983; Sewell, 1995; Sulkin & Pike, 1949, 1951; Sullivan et al., 1978). If the agent is suspected, such as *Mycobacterium tuberculosis* in a sputum specimen from a suspected TB patient, we should treat the specimen as if it did contain TB. Caution should always be exercised when working in the laboratory with known and well characterized agents that might have originated from epidemics, or that might be resistant to antimicrobial agents.

If the agent is known, but it is poorly characterized, the risk assessment process will be more difficult because of the lack of published information. Examples of such agents may be found in a 1997 review of infectious diseases by Mahy (1997) who listed 42 new viruses and

four new rickettsia discovered since 1988. Because the agent is known, information on related microbial family members and other published disease observations may be available. The clinical signs and symptoms of disease and inferences on transmission characteristics by medical practitioners may also have to be relied on.

The most difficult assessment occurs when the agent is unknown because there is little or no information available. Initial outbreaks of hantavirus infection (CDC, 1994) with pulmonary syndrome, and Hendra and Nipah viruses (CDC, 1999) are examples where the infecting agent was initially unknown and uncharacterized. Under these circumstances information from field outbreaks, and servicing medical practitioners, might be the only available information. Because so little is known about the transmissibility of these unknown agents naturally and in the laboratory, the risk to the laboratory worker is higher.

Pathogenicity

Pathogenicity is the ability of an agent to cause disease and varies with the subtype, strain, or antimicrobial resistance of the infectious agent. For example, there are pathogenic and nonpathogenic strains of Ebola virus, *Escherichia coli*, and *Bacillus anthracis* (anthrax). Ebola Zaire is highly pathogenic for humans whereas Ebola Reston does not appear to cause disease in humans although it does in primates. *Escherichia coli* is a normal member of our intestinal flora; however, *E. coli* strain 0157H7 is a deadly pathogen. The difference between pathogenic and nonpathogenic strains of anthrax is dependent on the presence of a plasmid in virulent strains. Many agents, such as *Clostridium botulinum,* depend on the secretion of toxins for their pathogenicity.

Virulence

Virulence is the degree of pathogenicity. Virulence depends on the infectivity of the agent and the severity of disease (which will be discussed in a following paragraph). Some disease isolates may require a lower infectious dose (discussed in a following paragraph) and will be more transmissible, more invasive, and more severe than other isolates. *Neisseria meningitidis* is endemic in our population, but epidemics occur and isolates from these epidemics might be more transmissible or more invasive than endemic strains. Endemic and epidemic strains of influenza would be another example. Virulence can also vary with the route of infection. When inhaled, anthrax spores can cause a fatal pneumonia, but if introduced into the skin the spores will cause a cutaneous lesion. Unless the virulence of the isolate or strain is known with certainty, it is best to assume that the strain is pathogenic and virulent.

Infectious Dose

It would be ideal to have accurate data on the infectious dose for every route of infection for every human pathogen. Unfortunately, infectious dose data for human pathogens, obtained from studies on human volunteers, are rare. Infectious dose data for human pathogens obtained from studies on experimental animals may or may not have close relevance to humans. Generally, those agents with the lowest infectious dose, such as Q fever with an infectious dose of 10 for humans by the aerosol route, Venezuelan equine encephalitis with an infectious dose of 1 for humans by the subcutaneous route, or measles with an infectious dose of 0.2 for humans by intranasal spray (Collins, 1983), are the greatest risks for laboratory transmission. Dilute samples of infectious agent with low infectious dose (e.g., 1-10 microorganisms) can be more hazardous than concentrated samples with a high infectious dose (e.g., 1,000-5,000 microorganisms). Agents with low infectious doses, therefore, pose a higher risk of transmission. When this information is available, it may be very valuable in the risk assessment process.

Severity of Disease

The more severe the infectious disease the higher the risk for the laboratory worker and usually the higher the biological safety level for working with the agent. *Staphylococcus aureus*, which can normally be found on human skin, can also cause a wide variety of diseases in humans which are generally moderate in nature and can usually be treated with antibiotics, is classified as a BSL-2 agent. Although anthrax can cause a severe to lethal disease in humans and can be transmitted by the aerosol route, it is classified as a BSL-2 agent when working with clinical specimens and

as a BSL-3 agent when working with the purified agent because of its susceptibility to antibiotics. Ebola virus, which may also be transmitted by the aerosol route in the laboratory, can cause lethal disease (up to 90% mortality) for which there is no known effective treatment and is classified as a BSL-4 agent. On the other hand, strict bloodborne pathogens which can also be lethal, such as HIV and HBV, are classified as BSL-2 agents because of their lack of transmissibility by the aerosol route.

Regulatory Requirements

Regulatory requirements for working with infectious agents in the laboratory (as opposed to transportation) are based on the institutional, geographical, and host origins of the agent. If the agent or the material that contains it originates in a foreign country and it is a human pathogen, it will require an import permit from the CDC (USDHHS, 1985; Richmond & McKinney, 1999). If the agent is a livestock or poultry pathogen, it will require a permit from USDA/APHIS (USDA, 1999) to import it and any materials that might contain it and to transfer it domestically. The domestic transfer of a number of human agents and toxins, termed select agents, requires that transferring facilities be registered with the CDC and, when transferred, CDC must be notified (USDHHS, 1994). The regulatory requirements usually specify biosafety levels for working with the agents and may specify specific handling, transfer, and disposal requirements. The Occupational Safety and Health Administration has also specified practices and procedures for working with bloodborne pathogens in the laboratory (USDOL, 1991).

Host Range

Each infectious agent has a unique host range which may vary from a single host to a wide variety of human, animal, and insect hosts. The only known reservoir for *Neisseria meningitidis*, for example, is humans. On the other hand, Venezuelan equine encephalitis (VEE) virus is pathogenic for humans, horses, and laboratory animals and is borne by several different mosquito vectors. A wide host range may necessitate additional prevention measures. Because of its wide species range, laboratories and particularly animal facilities containing VEE-infected animals must have a strict mosquito control program in effect. Furthermore, because VEE is a pathogen for horses it is a "restricted animal pathogen" and requires a USDA/APHIS permit to import it or transfer it domestically.

The Laboratory Sample

The previous paragraphs have discussed the characteristics of the agent. However, the laboratory worker encounters the agent in the laboratory in the form of a sample or a series of samples used in purifying, concentrating, and identifying the agent. The sample may be a clinical specimen composed of body fluids such as serum, blood, urine, and tissues, or liquid or agar cultures of a bacteria, or viral cell cultures. As the laboratory worker works with the sample, the nature, concentration, and volume of the sample may change. For example, a clinical specimen is inoculated into broth cultures and then streaked on agar plates. Purified colonies are scraped off the plates, concentrated in a centrifuge, resuspended in buffer, and then pipetted into microculture plates.

As the sample proceeds through the work process, the degree of risk to the worker changes as the nature of the sample changes. Clinical samples of blood, serum, or tissue are likely to contain lower concentrations of an infectious agent and consequently have a lowered risk of transmission. Purified, concentrated cultures of bacteria or viruses in liquid solutions pose the highest transmission risk of all because if dropped, pipetted, vortexed, or spilled the liquid material would generate splashes, splatters, micro droplets, and aerosol particles loaded with infectious particles. In general, the higher the concentration of the agent in the sample, the larger the volume of the sample; and the easier it is to aerosolize the sample, the higher the risk for the worker. The BMBL uses this concept to recommend work with clinical specimens of a number of agents such as *Neisseria meningitidis* to be performed at BSL-2, whereas work with concentrated agent preparations is recommended for BSL-3.

Laboratory Animals

The risks associated with laboratory animals are in proportion to the degree to which the infected animal can transmit the agent by bite or scratch, urine, feces, or by contaminated bedding or water. For example, working with experimentally infected rodent species not known to excrete the causative agent of hantavirus with pulmonary syndrome (HPS) provides a lower risk for the worker and can be performed at Animal Biosafety Level-2 (ABSL-2). On the other hand, aerosols from contaminated bedding from experimentally infected deer mice of the genera *Peromyscus maniculatus*, which are the natural hosts of HPS, should be considered a much higher risk and work should be conducted at ABSL-4 (CDC, 1994).

Insect Vectors

Insects provide a different kind of risk. Care must be taken to protect experimentally infected animals in animal facilities from biting insects that can transmit the disease to humans or other animals. Fleas, for example, can transmit *Yersinia pestis*, the causative agent of plague, from rats to humans. Mosquitoes can transmit many arboviruses from infected animals to humans. When working with insect-borne diseases in experimental animals, the animal facility must have a vigorously enforced insect control program.

Experimentally infected insects, such as malaria-infected mosquitoes, when worked with in laboratories or insectories, can escape, and bite the handler or other workers. This work requires special containment processes, such as netting, insect traps, and special practices and procedures, such as anesthesizing and counting the mosquitoes, to reduce the risk of being bitten. At least two cases of malaria transmitted from mosquitoes to humans in research insect containment facilities have been reported to the author.

Transmission Potential of Laboratory Activity

Can the agent be transmitted from the laboratory sample to the worker by aerosol, ingestion, mucocutaneous exposure, or parenteral inoculation? Aerosols are considered the most hazardous mode for infectious agents because of the large number of personnel that can be infected by this route, and because most laboratory-acquired infections are known or suspected to have been caused by aerosols. Most laboratory activities, such as pipetting liquid material, shaking or vortexing containers, grinding tissues in blenders, using improper centrifuge procedures, streaking agar plates and breaking of any culture containers, have the potential for generating aerosols (Collins, 1983). Any agent that can cause upper respiratory disease, such as *Mycobacterium tuberculosis* and influenza, is a likely candidate for aerosol transmission. *Shigella dysenteriae* is transmitted in nature by ingestion of contaminated food or water and ingestion would also be the major hazard of this agent in the laboratory. On the other hand, diseases such as VEE or Rift Valley Fever that are transmitted in nature by insects are highly transmissible to man by the aerosol route when working with viral samples in the laboratory. Other agents such as human immunodeficiency virus (HIV) or hepatitis B virus (HBV) are poorly transmitted or not transmitted at all by the aerosol route, either in nature or in the laboratory.

Other routes of transmission must also be considered. Splashes and splatters could come in contact with the mucous membranes of eyes, nose, and mouth, or open cuts and wounds. Contamination of laboratory surfaces can contaminate hands, which in turn can lead to infection by mucocutaneous contact or ingestion. Contaminated sharps, such as broken glass, scalpels, and needles, could transmit an agent parenterally. Eating, drinking, or smoking in the laboratory could lead to inadvertent ingestion of an agent.

Susceptible Route

To infect a laboratory worker the agent must be transmitted from the animal or insect source or the laboratory sample in a form that can gain entry to the worker by a susceptible route. The susceptible route is dependent on the infecting agent. Some infectious agents may be transmitted by multiple routes whereas others may be restricted to one or two routes. *Plasmodium vivax*, one of the causative agents of malaria, is a bloodborne pathogen and can only be transmitted by the parenteral route through mosquito bites, needles, or sharps. Some enteric pathogens such as *Salmonella typhi* are naturally transmitted by ingestion but could

be transmitted parenterally in the laboratory by sharps. Many of the arboviruses, such as VEE, which require an insect vector for parenteral transmission in nature, may nevertheless be accidentally transmitted in the laboratory by aerosols generated from lab operations.

Unless otherwise carefully documented, it is best to assume that agents can be transmitted by multiple routes.

Susceptible Host

Although an agent may gain access to the body through the appropriate portal of entry the host must also be susceptible. Susceptibility to an agent depends on a number of factors ranging from the age of the host, current health, to the immune status of the host. A laboratory worker in good health will generally be more resistant to an infectious disease, and if infected will recover more quickly. Young children, the elderly and those workers with chronic diseases are at higher risk of acquiring infectious diseases. Workers suffering from immunosuppressive diseases or who are treated with immunosuppressive drugs are also at increased risk of acquiring infections in the laboratory and suffering a more severe form of disease than the nonimmunosuppressed person.

Prophylaxis

The availability of effective prophylactic measures reduces the worker's risk of acquiring a laboratory infection. The most effective way to reduce susceptibility to an agent is through vaccination. Unfortunately, vaccines are available for only 20 or so of the hundreds of infectious diseases. When available they must be offered to the laboratory worker. However, the limitations of vaccines also need to be understood. Immunity after vaccination declines over a period of time, necessitating a surveillance program that includes periodic revaccinations. Some vaccines require multiple doses over a period of months to stimulate an immune response, and immunity after the first few doses may not be complete. Vaccines such as the influenza vaccines are highly specific for the vaccination strain and may not protect against recent epidemic strains imported from distant outbreaks.

In some cases, such as potential exposures to Hepatitis B virus, it is also possible to boost the resistance of the host by administering specific immune serum globulin. Antibiotics and antivirals also have the potential for being used for prophylactic purposes, although this is rare. During the recent avian influenza outbreak in China, laboratory workers in the U.S. working on clinical samples of influenza isolates from the outbreak in China were offered the anti-influenzal agent Rimantidine for additional protection.

Treatment

The availability of effective treatment after an exposure reduces the risk to the worker. Specific immune serum immunoglobulin can be given to provide immediate protection to a worker for an exposure to HBV while concurrently vaccinating the worker. Antibiotics are available for most bacterial, rickettsial, and fungal agents, anti-viral chemotherapeutic agents for selected viral agents, and chemotherapeutic agents for many protozoan and parasitic diseases. Generally, treatment is most effective if given as soon as possible after the exposure to the infectious agent to minimize its replicability and to buy time for the immune response to develop. Zidovudine, for example, optimally should be given within several hours after exposure to HIV (CDC, 1998).

Skill Level

The degree of knowledge and experience in working with the agent in the laboratory environment and the amount of biological safety training and experience with specific safety work practices influence the level of risk. A knowledgeable and well trained laboratory worker is at a much lower risk of contracting a laboratory-acquired infection than one who isn't. As the BMBL emphasizes, the higher the biosafety level of the agent, the higher the required skill and experience level of the worker and supervisor.

Recombinant DNA Microorganisms and Products

The ability to excise one or more genes from one source of animal, plant, or microbial DNA and to transfer the excised genes into another living organism,

while offering many possibilities of benefiting mankind also offers the possibility of creating new biohazards. One type of biohazard involves transferring gene coding for such characteristics as antibiotic or antiviral resistance, virulence factors, or toxins to other microorgansims, thus increasing their virulence. Other transferred genes may alter the host range, cell tropism, or cell cycle. Another possibility is that nonharmful genes in one host may inadvertently become harmful when transferred to another host. The growing use of viral vectors to introduce genetic elements into plants, animals, and humans also offers possibilities of unforeseen biological hazards. Risk assessments for such potential biological hazards must be reviewed by an Institutional Biological Safety Committee as indicated in the National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules (USDHHS, 1994).

Risk Assessment and the Biological Safety Program

The risk assessment process is an essential part of an effective biological safety program. The laboratory worker should be knowledgeable about every risk factor for the biological agents commonly used or encountered in the laboratory. Information for these risk assessments is best contained in a biological safety manual. Evaluation of each risk factor for an agent should lead to a risk management procedure. For example, for agents that are transmissible by the aerosol route, every effort should be made to minimize the generation and release of aerosols by using procedures such as using plastic containers instead of (breakable) glass, putting safety cups on centrifuges, confining work with the agent to biosafety cabinets, and using facilities with single passage directional air flow.

Summary

The BMBL provides agent summary statements for more than 100 agents. These agent summary statements provide much of the necessary information for evaluating essential risk factors. However, new and emerging and reemerging agents are continually being encountered. Mahy (1997) has described 40 new viral and rickettsial agents since 1988. Many of these remain poorly described and characterized. Other agents may have developed resistance to antibiotics and chemotherapeutic agents. The host range of many agents, such as Nipah virus and bat lyssaviruses, are not fully known. Genetic engineering offers the possibility of modifying many agents by adding or deleting genes that may further modify the pathogenicty of the agent and create new biohazards or using r-DNA vectors to insert genes into host cell DNAs either *in vitro* or *in vivo*. It is these new and emerging agents, for which information is often scarce, for which the risk assessment process is most valuable and the use of the risk factors described herein will hopefully find use in ensuring safe work with these agents in the workplace.

References

Benenson, A. S. (Ed.) (1995). *Control of communicable diseases manual.* (16th ed.). Washington, DC: American Public Health Association.

CDC. (1999). Outbreak of Hendra-like virus—Malaysia and Singapore 1998-99. *Morbidity and Mortality Weekly Reports, 48*, 265-269.

CDC. (1994). Laboratory management of agents associated with Hantavirus Pulmonary Syndrome: Interim biosafety guidelines. *Morbidity and Mortality Weekly Reports, 43*, 1-7.

CDC. (1998). Guidelines for the use of anti-retroviral agents in HIV-infected adults and adolescents. *Morbidity and Mortality Weekly Reports, 47*, RR-5.

Collins, C. H. (1983). *Laboratory-acquired infections: History, incidence, causes and prevention.* Butterworths, and Co. Ltd.: Oxford, England.

Kennedy, M. E. (Ed.). *Laboratory biosafety guidelines.* Ottawa, Ontario, Canada: Laboratory Centre for Disease Control, Health Canada.

Knudsen, R. C. (1999). Risk assessment for biological agents in the laboratory. *Journal of the American Biological Safety Association, 3*, 99-104.

Kruse, R. H., Pucket, W. H., & Richardson, J. H. (1991). Biological safety cabinetry. *Clinical Microbiology Reviews, 4*, 207-241.

Mahy, B. W. J. (1997). Why are new virus diseases emerging and re-emerging? *PHLS Microbiology Digest, 14*, 135-137.

Richmond, J. Y., & McKinney, R. W. (Eds.) (1999). *Biosafety in microbiological and biomedical laboratories.* (4th ed.) United States Department of Health and Human Services: Washington, DC.

Sewell, D. L. (1995). Laboratory associated infections and biosafety. *Clinical Microbiology Reviews, 8*, 389-405.

Sulkin, S. E., & Pike, R. M. (1949). Viral Infections contracted in the laboratory. *New England Journal of Medicine, 241*, 205-213.

Sulkin, S. E., & Pike, R. M. (1951). Survey of laboratory acquired infections. *American Journal of Public Health, 41*, 769-781.

Sullivan J. F., Songer, J. R., & Estrem, I. E. (1978). Laboratory acquired infections at the National Animal Disease Center, 1960-1975. *Health and Laboratory Sciences, 15*, 58-64.

USDA. (1999). Animal and Plant Health Inspection Service. 9 CFR Part 122. Organisms and Vectors.

USDHHS. NIH guidelines for research involving recombinant DNA molecules. *Federal Register, 59*(127), 34495-34547.

USDHHS. (1996). Public Health Service. 42 CFR Part 72.6 Additional requirements for facilities transferring or receiving select agents. *Federal Register, 61*, 55190-55200.

USDHHS. (1985). Public Health Service. Part 71. Foreign Quarantine. Etiologic Agents and Vectors. *Federal Register, 50*, 1224-1228.

USDOL. (1991). Occupational Health and Safety Administration: Occupational exposure to bloodborne pathogens: Final rule. *Federal Register, 56*, 64175-64182.

Editor's note: This chapter is reprinted from *Anthology of Biosafety III: Applications of Principles*, pp. 1-10. ©2000. Reprinted with permission of the publisher, ABSA, and the Editor, Jonathan Y. Richmond.