

RISK ASSESSMENT FOR BIOLOGICAL AGENTS IN THE LABORATORY

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ABSTRACT

Risk assessments for using biological agents in the laboratory are qualitative rather than quantitative. A number of risk factors for performing the assessment are identified and discussed in terms of information needed and assessment. A process for performing a qualitative risk assessment for biological agents in the laboratory is presented with an example.

INTRODUCTION

Infectious agents of humans, when worked with in the laboratory environment, are hazards, or risks, to laboratory workers, their co-workers, their families and their communities. Laboratory work includes working with known, suspected, or unknown infectious agents in clinical or diagnostic specimens, or working with specific agents in purified and concentrated form for either diagnostic or research purposes. Laboratory work also includes working with a variety of normal as well as infected animals or their tissues in either diagnostic or research situations. Risk assessment is the process of evaluating the risk to the laboratory worker when working with the infectious agent in the laboratory environment and assigning the biosafety level (i.e., the facilities, equipment and practices) that will reduce to an absolute minimum the risk of exposure of the worker to the agent.

Biosafety Levels

The CDC/NIH guidelines Biosafety in Microbiological and Biomedical Laboratories (BMBL) (6) has defined four biosafety levels (BSLs), termed BSL 1, 2, 3, and 4, for working safely with any one of a variety of infectious agents of humans in laboratory

and animal facilities. Each biosafety level has specific facility, equipment and practice requirements for working with infectious agents at that level. A complete discussion of the four biosafety levels is found in the BMBL. They are discussed very briefly below:

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.

The Quantitative vs. Qualitative Risk Assessment

Risk assessments are either quantitative or qualitative. A quantitative risk assessment is directly dependent on the accurate measurement of the hazard, such as the concentration of a toxic gas, and associated information on the concentrations of the gas that are toxic for humans. An example of this would be the measurement of the concentration of residual formaldehyde gas present in BSL-4 laboratory after it has undergone a formaldehyde gas decontamination followed by neutralization and ventilation of the laboratory spaces to remove any residual gas. A value lower than 0.5 ppm indicates that the neutralization and subsequent ventilation was successful and that personnel can safely enter the laboratory.

However, risk assessments for working with infectious agents are usually qualitative because there are many unknown variables associated with the

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transmission of infectious agents for which quantitative data is either difficult or impossible to obtain. Infectious agents include a variety of microorganisms including bacteria, viruses, fungi, rickettsia, mycoplasma, protozoans, multicellular parasites, and prions. Each species of agent may also have types, subtypes and variants that differ from one another in virulence, host range, transmission, sensitivity to antimicrobial agents, etc. Infectious agents may be transmitted by a variety of methods such as aerosols, fomites, contact, and insects or other vectors. Current technologies also allow us to genetically engineer microorganisms by either transplanting a variety of genes, such as those for virulence and antimicrobial resistance, or to delete genes in some agents to reduce their virulence. In some instances we may be able to harness new technologies to develop novel vectors such as creating liposomes containing nucleic acids for injection into mammalian cells. Established cell lines may also harbor oncogenic viruses which may also pose a hazard to workers. It is this diversity of microorganisms, carcinogenic cells, genetic recombinants and unusual vectors and their multiple methods of transmission to susceptible hosts that makes it difficult to define unique chemical differences and meaningful sampling methods that might be exploited to detect them in quantitative assays.

Another limiting factor is the complex chemical composition of microorganisms. Infectious agents are complex mixtures of lipids, proteins, polysaccharides, nucleic acids in a milieu of smaller chemical and biochemical macro and micromolecules that provide many barriers towards using chemical methods of determining the identity and number of infectious particles in potential human exposure situations.

A further limitation is the degree of immune resistance of the laboratory worker to infection. In any group of people, some have a higher natural resistance to infectious agents than others. Some may have partial or complete immunity to the agent based on a past history of natural infection. Others may be completely protected against the agent after being vaccinated. Thus the range of a laboratory worker's susceptibility to disease when working with an infectious agent in the laboratory is directly dependent on the immune status of the worker and may vary considerably from worker to worker.

However, the major limitation to quantitative measurement is that infectious agents need to be measured on the basis of their reproductive capacity. An infectious agent that can reproduce in the host is a viable agent. However, a viable infectious agent may not necessarily reproduce in the host. For both the disease process and the assay process the microbe, after infection, must be able to reproduce itself many times over before it can damage the host. In this sense we must view the capacity of an infectious microbial cell to amplify itself in the host as its most significant characteristic. For example, if a person is infected with 10 to 100 infectious bacteria of *Mycobacterium tuberculosis* it is not this small number that will harm the host. It is their progeny, amplified more than a million or a billion times, that will make the host sick. If the microbe cannot amplify itself in the host, it will not make the host sick. Quantitative in vitro assays, if they are to be meaningful in evaluating transmission of the agent to the host, need to be based on measuring the microbe as an amplification unit, which requires measuring the reproductive capacity of the agent and hence its viability. Unfortunately, assays based on measuring the agent as an amplification unit are, for a variety of reasons, technically difficult to perform and interpret and are mostly used in special research situations under carefully defined environmental parameters with only a few carefully selected and well characterized microbes.

It is the nature of infectious microbes, their diversity, their complex composition, their wide variety of transmission methods, and their capability to reproduce in the host during the disease process, that makes it difficult with current technologies to develop rapid, sensitive, quantitative assays of infectious agents as exposure hazards. In the absence of quantitative measures we must rely on qualitative measures.

Performing the Qualitative Risk Assessment

In performing a risk assessment for biological agents in laboratory situations it is first necessary to identify all of the risk factors that could be involved in transmitting the agent to the lab worker under laboratory conditions. Information for each risk factor must be collected and evaluated. Information may be published or unpublished. Published data would include agent summary statements published

in the BMBL (6), lists of agents and their biosafety levels such as those published in the NIH's Recombinant DNA Guidelines (5) or the Canadian Laboratory Biosafety Guidelines (3), research papers on transmissions, reviews of laboratory acquired infections (2, 4, 7, 8, 9, 10) etc. Unpublished data may come from subject matter experts and epidemiologists and other scientists in field situations. Information for each factor needs to be interpreted for its tendency to increase or decrease the risk assessment, and hence the biosafety level. The final step is to evaluate all of the risk factors together before assigning a biosafety level for working with the agent.

Risk Factors, Related Information and Their Evaluation

Table 1 lists a number of risk factors, the types of information necessary to evaluate the risk factor, and an indicator system of directional arrow points that indicates that the assessment for the factor should be increased or decreased.

Agent Identity. The first risk factor involves establishing the identity of the agent. If the agent is known or its identity is strongly suspected, for example *Mycobacterium tuberculosis*, the BMBL may be used to determine the biosafety level. However, a number of agents, such as Epstein-Barr virus, are not listed in the BMBL. In this situation other published information needs to be collected. A good source of disease transmission information is available for a wide variety of agents in the American Public Health Association Control of Communicable Diseases Manual (1). Other sources of information may be found in the scientific literature such as literature reviews on laboratory acquired infections (2, 6, 7, 8, 9, 10).

In some cases we may be dealing with clinical specimens or other samples from an outbreak with a known agent such as the marine dinoflagellate *Pfiesteria piscacida*, previously unsuspected as a human pathogen, which suddenly becomes linked with pathogenic properties during the outbreak. In other cases we may be dealing with samples from a disease outbreak where an infectious agent is suspected but the identity is completely unknown. An example of this is the hantavirus outbreak in the four corners area of New Mexico, Arizona, Colorado and Utah which resulted in the death of 17 people before the agent was identified. In this

situation, information on the signs, symptoms, pathology, and suspected transmission modes will be needed from epidemiologists, physicians, scientists and health care workers dealing with the outbreak in field situations.

Agent Transmission. The second risk factor involves determining the transmission mode. Infectious agents may be transmitted by inhalation through the respiratory tract, ingestion, contact with mucous membranes, or parenterally by sharps, animal bites or insect bites. Transmission is dependent on the type of sample that is being worked with, the laboratory activity that is being performed with the sample, and the possible routes by which the agent may infect the worker.

In the laboratory environment the majority of laboratory acquired infections appear to have been transmitted by the aerosol route. The aerosol route is the hardest route of infection to control and is therefore the most important to evaluate in the risk assessment. Indeed, BSL-3 and 4 facilities are specially constructed to contain aerosols of infectious agents, and the biosafety cabinet was developed to contain aerosols.

The aerosol potential of a sample is dependent on the concentration of the agent per unit volume in the sample and on the laboratory activity that is being performed with the sample. Of the samples dealt with in the laboratory, clinical specimens of blood and other tissues usually contain the lowest number of microbes. Because these samples also tend to be viscous they reduce the transmission hazard from aerosol to splatters and splashes. The aerosol risk increases as we amplify the agents on growth media or in cell cultures and subsequently concentrate them further by centrifugation. In addition, we need to evaluate the laboratory activity. Streaking a sputum sample on an agar plate carries less aerosol risk than grinding tissues in a homogenizer or concentrating viruses from cell cultures in an ultracentrifuge. The greater the aerosol potential, the greater the higher the assessment.

The second aspect of transmission is related to the route by which the agent can infect the worker. Some infectious agents, such as *Mycobacterium tuberculosis*, are highly infectious by the respiratory route, but are far less infectious by other routes such as ingestion, contact with mucous membranes or parenteral routes. Other agents, such as *Plasmodium falciparum*, are highly infectious by the par-

TABLE 1
Qualitative Risk Assessment of an Infectious Agent

Risk Factor	Data Collection	Assessment Decrease<<<=>>>Increase
1. Agent identity		
Known, classified	Agent summary statement	<=>
Suspected, classified	" " "	<=>
Known, unclassified	Disease Information	=>>>
Unknown	" " "	=>>>
2. Agent Transmission		
Aerosol potential (Sample/activity)	Tissue/activity	<<<=
	Culture/activity	=>>>
	Concentrate/activity	=>>>>>
	Animal/non-shedder	<<<=
	Animal/shedder	=>>>>>
Infectious route	Respiratory	=>>>>>
	Mucous membrane	=>>>
	Parenteral	<<<=
	Other	<<<=
3. Disease Severity		
	Moderate	<<<=
	Severe	=>>>
	Life threatening/lethal	=>>>>>>>
4. Prophylaxis		
	None	=>>>>>>>
	Vaccine	<<<=
	Ig	<<<=
	Antibiotics	<<<=
	Antivirals	<<<=
5. Treatment		
	None	=>>>>>>>
	Other	<<<=
6. Other		
Livestock pathogen	USDA regulations	=>>>
Poultry pathogen	USDA regulations	=>>>

enteral route but far less infectious or noninfectious by other routes. Because of the known capability of *M. tuberculosis* to be transmitted by aerosols laboratory work with this agent is performed at BSL-3 whereas work with *P. falciparum* is performed at BSL-2.

Laboratory animals also pose a variety of transmission hazards. Animals may excrete infectious agents in urine and feces, may disseminate agents by the respiratory route, and may inflict bites and scratches on the handler. Animals that shed virus through respiratory dissemination or dissemination in urine or feces are far more hazardous than animals that do not.

Disease Severity. The more severe the disease that might be acquired by the laboratory worker the higher the assessment. *Staphylococcus aureus* only rarely causes a severe or life threatening disease in a laboratory situation and is classified as a BSL-2 agent. Viruses such as Ebola, Marburg and Lassa fever, which cause diseases with high mortality rates for which there are no vaccines or treatment, are worked with at BSL-4. However, human immunodeficiency virus and hepatitis B virus, which can cause life threatening and lethal disease, but which are not transmitted by the aerosol route, can be worked with at BSL-2.

Prophylaxis. The risk of working with infectious agents decreases if effective prophylactic measures are available. The most common prophylactic measure is vaccination. The classification of work with Junin virus in the BMBL illustrates the importance of vaccination in setting biosafety levels. Junin virus is classified as a BSL-4 agent in the BMBL. However, workers immunized with Junin vaccine may work with this agent at BSL-3. Other forms of prophylactic measures are used more rarely. The administration of specific immune serum globulin may be used for hepatitis B virus. There may be special circumstances where prophylactic administration of antibiotics and antivirals may be used. Rimantidine was administered prophylactically to some lab workers in influenza laboratories during the recent "bird flu" outbreak in Hong Kong.

Treatment. In similar fashion, the risk of working with infectious agents decreases if effective treatment is available. Effective treatments include the use of vaccines, specific immune globulin, antibiotics and antivirals.

Other Risk Factors. In addition to the major risk factors discussed above, there are a number of other risk factors that need to be considered at various times and in various situations. Only a few will be discussed here as examples. If the infectious agent is a zoonotic agent it may also be of danger to the American livestock and poultry industry and as such comes under the jurisdiction of the United States Department of Agriculture, Animal and Health Inspection Service (USDA/APHIS) which tends to increase the risk assessment. Other factors to be considered here might be antibiotic or antiviral resistance, genetically engineered microorganisms, novel vectors such as liposomes containing nucleic acid material, and agents, such as smallpox virus, that have been eradicated from human populations and exist only in the laboratory environment.

Assessing the Biosafety Level for *Neisseria meningitis* Through Risk Assessment

An example of a qualitative risk assessment for laboratory work with *Neisseria Meningitis* is provided in the following paragraphs and in Table 2.

Laboratory work: The laboratory has received isolates of several epidemic strains of *Neisseria meningitis* Group B, for which no vaccine is available, from foreign laboratories. The laboratory activities will involve expanding these cultures on agar slants, preparing suspensions in phosphate buffered saline, measuring the optical density of the suspensions in a spectrophotometer, and with an eight channel micropipeter, dispensing the suspension into microculture plates for microscopic analysis. The agent summary statement is found on page 97 of the BMBL.

Assessment: The risk assessment for each factor is found in Table 2. The agent identity is known and can be found in the BMBL. The laboratory activities would generate aerosols as well as splatters and splashes and the agent is infectious to humans by the aerosol route. The disease caused by these agents, which are epidemic strains, are severe to life threatening in the absence of antibiotic therapy. An effective vaccine is not available, although if exposed, laboratory workers can be effectively treated with antibiotics. The described laboratory work with these agents should be performed at BSL-3.

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TABLE 2
Assessing the Risk for Epidemic Isolates of Group B *Neisseria meningitidis*.

Risk Factor	Assessment Decrease<<<=>>>Increase
1. Agent Identity	
Known, classified	<<<=
2. Agent transmission	
Aerosol potential	=>>>
Infectious route	=>>>
3. Disease Severity	
Severe	=>>>
4. Prophylaxis	
(None)	=>>>>>>
5. Treatment	
Antibiotics	<<<=
6. Other	
none	Not applicable