

Appropriate Containment for Opportunistic and Borderline Pathogens

Policy of the Institutional Biosafety Committee

University of Wisconsin - Madison

Introduction

A good starting point for risk assessments and determination of appropriate precautions is the risk group (RG) listing of pathogens provided in the NIH *Guidelines* (Appendix B). Included in this listing are representative genera and species that are known to be pathogenic. Microorganisms that normally do not cause disease in healthy human adults are categorized as Risk Group 1 and are not explicitly listed. The next step up in hazardous nature is Risk Group 2; these organisms have the ability to cause disease in healthy human adults but the infections typically are not severe and are treatable.

Agents that are not listed in Appendix B, however, are not automatically or implicitly classified in RG1. The listing is not meant to be all-inclusive and it does not include normal flora of humans that are pathogenic when they gain access to other than normal sites, such as might happen during a laboratory accident. Work with microorganisms in the laboratory setting may create situations whereby the normal route of transmission is circumvented. In the lab, the concentration and volume are typically higher than encountered outside of the lab and procedures provide “opportunities” to infect in an abnormal manner such as splash to mucosa or needle stick injury. Decisions regarding precautions for handling such organisms should be based on a risk assessment that considers the laboratory activities.

Microbes do not naturally segregate into discrete risk group categories but cover the spectrum of pathogenicity. Certain opportunistic pathogens, although ubiquitous in the environment or normally commensal, may cause significant disease in individuals whose immune system is stressed. The immunocompromised condition is not uncommon in our population and the principal investigator cannot reliably expect individuals to inform them of a change in their health status. This change may occur for many reasons, including routine illness, pregnancy, and chemotherapy to treat underlying illness. The IBC believes it is important to protect these individuals.

Some microbes seem to fall between RG1 and RG2. Risk assessments are needed that evaluate the pathogenicity of specific strains and the lab procedures involving such borderline pathogens. Clinical isolates are generally of greater concern than those collected from environmental samples.

Statement of Policy

Based on risk assessments, the IBC has determined that biosafety level 2 (BSL-2) precautions are appropriate for handling the following microorganisms:

Adeno-associated virus (AAV) – The wildtype virus is generally considered RG1 because it usually requires another virus for replication. However, BSL-2 precautions are appropriate because this virus can become latent by integrating into the genome and be expressed during infections with helper viruses. There also is some evidence of AAV infection of the human embryo in pregnancy and AAV has been associated with male infertility. The gutted AAV vector (e.g., missing the *rep* and *cap* genes) shows limited biodistribution in animal studies and treated animals may be handled using BSL-1 precautions if the transgenes are not likely to enhance pathogenicity. BSL-1 laboratory precautions are acceptable for gutted AAV vectors unless higher hazard transgenes (e.g., oncogenes) are expressed or high titers and/or large volumes of virus are handled, in which case BSL-2 should be used.

Aspergillus flavus and *A. fumigatus* cause opportunistic infections in the immunocompromised individual.

Bacillus anthracis (Sterne) is a vaccine strain and is missing an important virulence factor. There are some reports of pathogenicity of this strain in animals. Furthermore, per guidance from CDC, BSL-2 practices will avoid contamination of the lab, which could cause problems in confounding detection of anthrax.

Bacillus cereus - This foodborne pathogen produces toxin and causes cutaneous infections in healthy adults.

Candida albicans is an opportunistic pathogen with significant consequences for individuals with stressed immune systems.

Clostridium difficile - The “new” epidemic strain (B1/NAP-1) of this bacterium has substantial clinical consequences, is usually resistant to some antibiotics such as fluoroquinolones, and can infect healthy adults.

Enterococcus faecalis and *E. faecium* - Vancomycin resistant strains because vancomycin is an antibiotic of last resort. Furthermore, containment will minimize contamination of surfaces in the lab, which is important because this pathogen is extremely hardy in its ability to survive for weeks on environmental surfaces.

Pneumocystis jirovecii (*P. carinii* f.sp. *hominis*) causes opportunistic infections in the immunocompromised individual.

Pseudomonas aeruginosa – This pathogen causes chronic respiratory infections among cystic fibrosis patients and eye infections, especially in contact lens wearers.

Salmonella typhimurium LT2 - There is evidence that this strain may cause disease in healthy adults as well as immunocompromised individuals.

Stenotrophomonas maltophilia – This opportunistic pathogen has been implicated in infrequent ocular infections, primarily in patients with ocular compromise. Of particular concern is that treatment is limited by resistance to common antibiotics.

Additional information

Most wet labs will meet the standards for a BSL-2 facility but there are several key differences in practices between biosafety level 1 and 2. Some of the practices utilized under BSL-2 containment are:

- Biohazard signs are posted on the door to the laboratory when work with the microorganism is in progress.
- Individuals who may be exposed are informed of the potential risks to their health posed by the pathogen.
- Exposures are treated with first aid and reported to the PI with medical evaluation as deemed prudent.
- The lab is under negative air pressure relative to the corridor and the lab door is kept closed to maintain negative pressure.
- Many procedures can be conducted at the open lab bench under BSL-2 containment, but activities involving high concentrations and/or large volumes or that generate aerosols need to be avoided or done in containment.
- Use of a biological safety cabinet is preferred but is not required; alternative containment equipment may be acceptable.
- Disinfectants are chosen that are effective against the pathogens handled.
- Submission of a biosafety protocol is required.

Additional details concerning what working at BSL-2 entails can be found in the references below. Assistance is available from the Office of Biological Safety (263-2037; biosafety@fpm.wisc.edu).

References for guidance on BSL-2 precautions:

Biohazard Recognition and Control, UW-Madison biosafety manual

Biosafety in Microbiological and Biomedical Laboratories, CDC/NIH 5th ed.

Guidelines for Activities Involving Recombinant DNA Molecules, Appendix G, NIH

This policy was adopted by the IBC on 12/7/05 and amended 2/1/06 and 6/6/07. It will be reviewed and updated periodically.