

BioSide Lines

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Ethidium Bromide: Alternatives and Safe Handling

Ethidium bromide (EtBr) is a powerful mutagen that is commonly used to visualize nucleic acids. Ethidium bromide causes mutations by inserting between adjacent base pairs in a DNA molecule. Ethidium bromide is positive for a number of types of mutation assays: Ames (point mutations in bacteria), bacterial recombination, and mammalian cell culture (point mutations and recombinational mutations). UV transilluminators used for visualization of EtBr-stained gels are themselves hazardous because UV can cause eye and skin damage. Bleach is commonly used to "inactivate" EtBr prior to disposal but there is controversy regarding whether the resulting chemicals are themselves mutagenic.

Alternatives: Some alternative dyes are now available that are nontoxic or less mutagenic. Nile Blue is a relatively nontoxic alternative to EtBr. Although the sensitivity is approximately one fourth that of EtBr (S. Adkins, M. Burmeister, *Analytical Biochemistry*, 240:17-23, 1996), an added advantage is that visualization does not require UV light. This dye could be used for purposes that don't require great sensitivity (e.g., checking restriction enzyme digests to see if digests worked). Variables could be modified (e.g., longer staining times) to attempt to improve sensitivity.

SYBR Green is less mutagenic than EtBr in the Ames mutation assay. SYBR Green and other dyes such as Gelstar and Vistra Green are more sensitive than EtBr so handling is expected to be less hazardous because smaller amounts can be used. Dyes such as these that bind DNA, however, are expected to have some mutagenicity.

A nonmutagenic alternative that has similar sensitivity to EtBr is zinc-imidazole (E. Hardy et al., *Electrophoresis*, 17(1):26-9, 1996). Imidazole is not mutagenic but has high acute toxicity via inhalation, ingestion, and dermal contact routes. Zinc-imidazole could be an alternative to EtBr if staff wear appropriate protective equipment and only work with imidazole in a fume hood. Alternative chemicals that bind to zinc and are less hazardous could potentially be used in this method.

Safe Handling Tips: Always wear appropriate protective equipment when handling EtBr or other hazardous chemicals: lab coat (preferably Tyvek-type disposable), safety glasses, and double gloves. Latex gloves protect against EtBr but nitrile gloves protect against both EtBr and UV light. Use a UV-absorbing full face shield when working with a UV source that is not shielded.

Assign a designated area for use of EtBr, preferably in the fume hood. Work with concentrated amounts in the fume hood (also dilute, if possible). To minimize handling of powder, purchase EtBr as a pre-weighed tablet or as a stock solution. Staining after electrophoresis minimizes contamination of equipment. The EtBr staining solution can be used repeatedly, for several weeks (kept covered in the fume hood). Set up gel photography at or near staining area to minimize transport of EtBr-stained gels. Label designated areas where EtBr is used (e.g., mutagen) to communicate the hazard.

Contact Margy Lambert for more information or a copy of the article "An Eye for a Dye: Safe and sensitive new stains replace ethidium bromide for routine nucleic acid detection," (B. Sinclair, *The Scientist* 14(8):31, Apr.17, 2000).

Respiratory Protection: The Mask

People routinely tell us that they wear a “mask” to protect themselves from exposure to an inhalation hazard, but that term alone does not allow us to determine whether the protection is appropriate. Clear communication about the kind of respiratory protection used is critical since many kinds are available and selection depends on the type and degree of risk present. Wearing a respirator that is inappropriate for a given hazard may provide nothing more than a false sense of security.

Selection of a respirator to guard against pathogens is not as simple as for chemical hazards where tables of permissible exposure limits are available and background levels are factored into the decision. With the exception of clinical work involving TB, recommendations for respirators are not documented for pathogens since an acceptable exposure level has not been determined.

When considering exposure to microbes in the research laboratory, the background level should be negligible due to use of good microbiological techniques and a biological safety cabinet (BSC) when aerosol generation is unavoidable. Respiratory protection when handling pathogens is recommended for aerosol producing procedures that cannot be contained in a BSC, even for pathogens that normally are not transmitted via the aerosol route. Exposure to a high dose could overcome the natural barrier. Of particular concern is that some aerosol production is unanticipated and unintended, such as dropping the culture tube on the way to the BSC.

Filtering ability is a critical determinant of respiratory protection. Respirators that offer HEPA (high efficiency particulate air) filtration are the only kind that can adequately protect against the hazard presented by airborne microbes. The protection factor is improved by a full interior face seal. Look for the “NIOSH Approved” certification on the product.

There is a common misconception that non-HEPA filtered masks, such as dust and surgical masks, provide protection against infectious aerosols. Surgical and other common types of non-HEPA masks, generally distinguished by a single strap, provide poor filtration and poor fit.

Use of respiratory protection is prescribed for work that involves cultures of high hazard microbes, i.e., those categorized as risk group 3 having high individual risk but low community risk. While most activities are conducted in a BSC, contamination could be created without intent or warning such as in an emergency resulting from a spill. These researchers must have a medical evaluation, a fit test, and training in the proper use of the respirator.

An N95 mask is a reasonable choice to protect against exposure to allergens when handling animals and their bedding. The filtering material used in construction of the N95 mask is at least 95% efficient when tested with ~0.3 μm particles. N100 masks are only slightly better, 99.97% efficient. This efficiency is based solely on the materials used to construct the respirator and the protection actually afforded by the mask can be greatly reduced by poor fit. Facial hair, the shape of the face, strap tightness, head and face movements, and repeated use all can affect the seal and thus the protection factor. These problems with facepieces are alleviated by the use of full head cover with a powered air purifying unit.

The decision to use respiratory protection is highly judgmental. From a practical point of view, the decision could be made on the basis of whether an exposure of yourself and your coworkers is acceptable. From an employer's point of view, requiring workers to wear respiratory protection incurs significant costs, not only for the respirators but also for the medical evaluations and fit tests. Most researchers work under the circumstance where respiratory protection is available, possibly recommended, but not required, and this is a reasonable approach for most situations.

Basic Biosafety Class Offered

This class will give an overview of basic biological safety. Topics include: biosafety levels and biohazard containment, good microbiological techniques, waste disposal, risk assessment, and emergency preparedness. It is intended primarily for students and staff who are new to this institution and/or new to working with biological materials in a laboratory. Everyone is welcome to attend.

Thursday, May 22, 2003
Union South 1:30 – 3:00 p.m.

Registration is required. Contact Margy Lambert at 3-9013 or mlambert@fpm.wisc.edu.

Shipping Infectious Substances and Other Biological Materials

The Office of Biological Safety will provide training and certification for shipping Infectious Substance and other biological materials, with a focus on safety and regulatory compliance for research laboratories. The Department of Transportation requires that persons involved in shipping hazardous materials in commerce be trained and certified in proper handling of these materials.

Wednesday, April 9, 2003
Union South 1:30 to 3:30 p.m.
Refreshments will be served.

Registration is required. Contact Margy Lambert at 3-9013 or mlambert@fpm.wisc.edu

All staff are welcome to attend this class for initial or re-certification. Staff approaching their two-year expiration for certification will receive a notice in advance of that date. Computer-based training is available only for those who attended the class for their initial certification.

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