

BioSide Lines

October 2001

The Newsletter of the UW Office of Biological Safety

Heightened Precautions Recommended for Use of *Agrobacterium*

Agrobacterium tumefaciens is a common soil bacterium that causes plant crown gall tumors. This microbe is extensively used in plant biotechnology to transfer genes of interest to a wide variety of plants by taking advantage of its natural infection process. Some recent information warrants increased precautions for handling this microbe. Of particular concern is the recent evidence that *Agrobacterium* can infect and transform mammalian cells in culture.

Routine handling of recombinant *Agrobacterium* presumes that this bacterium does not infect humans. Traditional methods apply *Agrobacterium* to explants in plant tissue culture, a closed system that allows subsequent treatment to ensure removal of the bacteria. More recent innovations employ spraying the whole plant or dipping the inflorescence into a large volume of concentrated culture at the open bench, techniques that could allow its escape beyond the laboratory to the environment. The unintentional release of recombinant plant-associated organisms must be avoided, as is the case with all recombinant microbes.

Initially, *A. tumefaciens* was used to transform dicotyledonous plants. Techniques have now been developed for its extended use to transform monocots such as corn, and beyond the plant world to yeast and fungi.

A recent study presents evidence that *A. tumefaciens* can infect and transform human cells in culture. [Kunik et al. 2001. Genetic transformation of HeLa cells by *Agrobacterium*. PNAS 98: 1871-1876.]. This work was done with mammalian promoters; the incidence of transformation was increased when a plant-derived inducer was supplied. As of this time, however, there appears to be no further reference that confirms or extends this one report.

While there are a few reports in the literature of infection of immunocompromised individuals by *Agrobacterium*, it is not known to cause disease in healthy humans.

Based on these recent findings, the UW Institutional Biosafety Committee recommends adoption of the following sanitary containment practices in the laboratory and greenhouse for work with recombinant *Agrobacterium* and materials from infected plants:

- A biosafety cabinet, if available, should be used when aerosols are generated. We do not recommend use of a laminar flow clean air device for handling *Agrobacterium*.
- Use gloves when handling *Agrobacterium* and contaminated materials (plants and soil) especially if an open wound exists on hands.
- Collect and decontaminate residual plant water.
- Cleaning agents with demonstrated efficacy must be used to decontaminate surfaces and clean spills.

These recommendations about *A. tumefaciens* exemplify the need to be cautious with all microbes, including those that are deemed nonpathogenic for humans. Assumptions about what is considered safe are called into question as new information is revealed. Strains evolve and adapt to available niches. And recent studies show that traits are shared between distantly related microorganisms; minute gene sequence divergences have been shown to separate virulence and non-virulence. Thus, caution is advised.

Web-Access to Protocol Assurance Information

We did away with blue and yellow forms. Next on our target list is the venerable green form, which provides the assurance that a biosafety protocol is registered for the research covered by the grants listed. We had been told to keep it green because that makes it easier to find. However, wouldn't it better if you didn't need the paper copy at all? The Office of Biological Safety is edging into the age of electronic access to information. We believe this web-based process will allow us to better focus on conducting risk assessments, while making administrative information more readily available.

The information to be provided electronically includes the investigator's name and departmental affiliation(s), the general biosafety protocol title and expiration date, and the grant information that is associated with the protocol. The website will be updated monthly. Providing adequate security has been a critical consideration in developing this process. Didi Heisler and Jane Fox, Research & Sponsored Programs, have been instrumental in making it possible.

The process currently is undergoing testing but should be available soon to all departmental administrators who would like to have access. Call Biosafety (262-2037) if you would like electronic access to this administrative information. We will continue to provide paper assurances to principal investigators and others who request them.

Safety Glasses

The Safety Department recommends that staff always use protective eyewear when working in a hazardous environment, such as a laboratory where chemicals are handled and stored. Contact lenses may be used with discretion and in combination with eye protection, e.g., goggles or a face shield. These recommendations are based on the OSHA Lab Standard and *Prudent Practices for Handling Hazardous Chemicals In Laboratories* (National Acad. of Sciences). To help people be safe and comply, the Safety Department offers prescription safety glasses at minimal cost. Call Molly Stone, 262-8769, for more information.

Pipettes and Pipetting Aids

Pipetting is a common laboratory activity that can result in the production of aerosols and contamination of the surroundings. Aerosol production should always be minimized, even when working with nonpathogenic organisms. The unintentional release of recombinant microbes is unacceptable.

1. Never suction or pipette by mouth; always use some type of pipetting aid. Preferably, all activities with infectious agents should be confined to a biosafety cabinet (BSC) or other acceptable primary barrier. Pipetting of toxic chemicals should be performed in a chemical fume hood or, when product protection is necessary, in a class II-type BSC. Mouth pipetting should be prohibited even with devices that use an entire hydrophobic membrane filter not requiring fingers to touch the mouthpiece. This reusable pipetting device requires storage on the bench or other location between usage, which can result in contamination of the end piece that inserts into the mouth.
2. Infectious or toxic materials should never be forcefully expelled from a pipette. Mark-to-mark pipettes are preferable to other types, because they do not require expulsion of the last drop.
3. Infectious or toxic fluids should never be mixed by bubbling air from a pipette through the fluid.
4. Avoid mixing infectious or toxic materials by alternate suction and expulsion through a pipette.
5. Discharge from pipettes should be as close as possible to the fluid or agar level. The contents should be allowed to run down the wall of the tube or bottle whenever possible, not dropped from a height.
6. Pipettes used for transferring infectious or toxic materials should always be plugged with cotton, even when safety pipetting aids are used.
7. Avoid accidentally dropping infectious or toxic material from the pipette onto the work surface. Place a disinfectant dampened towel or other absorbent material on the work surface, and autoclave the towel before discard or reuse.
8. Contaminated pipettes should be placed horizontally in a pan or tray containing enough suitable disinfectant, such as a solution of hypochlorite*, to allow complete immersion of the pipettes. Pipettes should not be placed vertically in a cylinder that, because of its height, must be placed on the floor outside the cabinet. Removing contaminated pipettes from the BSC and placing them vertically in a cylinder provides an opportunity for contaminating the laboratory. An aerosol may be produced by displacement of contaminated air from inside a pipette during vertical insertion into a cylinder.

However, a tray containing the pipettes within the BSC can be covered and then removed and placed directly in the steam autoclave. **Note that hypochlorite should not be autoclaved.*

9. The pan and used pipettes should be autoclaved as a unit and replaced by a clean pan with fresh disinfectant.

Modified and reproduced with permission from Fleming, D. O. 1995. Laboratory biosafety practices. pp 203-218. IN Fleming et al (eds.) Laboratory Safety: Principles and Practices 2nd edition. ASM Press. Washington.

Shipping Infectious Substances and Other Biological Materials

The Office of Biological Safety will provide training and certification for shipping infectious substances 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, October 10, 2001

Union South 1 to 3 p.m.

Refreshments will be served.

Registration is required.

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

Staff approaching their two-year expiration for certification will receive a notice in advance of that date. You are welcome to attend the class. Computer-based training is now available as an alternative, but only for those who have attended the class for their original certification.