



Ask the Experts

John H. Keene

Biohaztec Associates, Inc., Midlothian, Virginia

In spite of all the good information available on the operation and use of biological safety cabinets (BSCs), it is evident that many who use them, regardless of their level of experience, simply do not understand either how they work or how to work in them. These are safety devices and it is our job as Biosafety Professionals to ensure that people who use them do so correctly. The question below seems to raise its ugly head over and over again. It recently appeared on BIO-SAFETY (a biosafety discussion list) and, in my opinion, the answer provided by Judy Pointer from M. D. Anderson Cancer Center (reprinted here with Judy's permission) hits the nail on the head.

Question:

A researcher claims that the use of a flame in the "tissue culture hood" (BSC) is necessary because the storage of trypsin/EDTA solution in plastic tubes has led to contamination in cultures. Consequently, the lab group uses glass bottles and flames the neck of the bottle before pouring trypsin into the tissue culture flask. A bottle of trypsin/EDTA is used repeatedly and stored in the freezer after each use. What methods should be used to ensure that the neck of the bottle is sterile?

Answer:

Tell the principal investigator (PI) that first of all, the contamination is probably coming from the multiple use of the trypsin—not the plastic or glass tubes. Once the contents of the tube/bottle are contaminated (glass or plastic), using that same vessel repeatedly only spreads the contamination. Preventing the container from being contaminated in the first place should be

the objective. How to do that without flaming the lips? **Use good sterile technique!**

First, the lab group should assess its system and the skills of those performing the work. The personnel may need more training, plus closer supervision. Second, they should run a test of their system. Select some sterile throwaway cell cultures. Remove the antibiotics in them and have each person in the lab carry the cultures for three weeks on routine maintenance (splitting, spinning, and replating). If they can't do this without contamination, they have one of two problems, 1) the cultures were seeded with low levels of contaminants from the start or 2) they are introducing them while working.

In my experience low levels of antibiotic-resistant common microbes (like *Pseudomonas*, yeast, and molds) were present in my cultures from the beginning. They were suppressed but never completely killed by the antibiotics. If this is the case, they need to figure out where the source is by isolating each step. My contaminants originally came from the incubator, the water bath, and a faulty Sweeny filtration apparatus I used for adding supplements. There were five different contaminants and each one was introduced a different way. None of the introductions was caused by "not flaming the lip of the bottle."

If, after finding the source, it is determined that contaminated bottle lips are the problem, the solution is easy. Don't touch the lip of the bottles with the pipette, and don't use the same, supposedly sterile, pipette repeatedly. If you do touch the lip, either throw the contents away and don't use it or carefully touch the drip on the lip with a sterile alcohol-soaked gauze pad. If you drop a lid, have sterile, prepacked ones available as replacements. If they are pouring the tryp-

sin out (bad technique in my opinion), they should be certain no media are on the lip before they pour.

What your tissue culturist doesn't realize is that laminar flow sterile cabinets are designed to work best when the airflow turbulence is at a minimum. That means, minimum movement, minimum temperature variations (heat from flames), and minimum introduction of contaminated fluids into the workspace. The introduction of wet media bottles from a contaminated water bath, or wet flasks or plates from a contaminated incubator can result in contamination of the cultures. The bottom line is—**no amount of flaming the lips of glass bottles will compensate for poor sterile technique.**

Note:

Trypsin is notorious for carrying contaminants. It is turbid and cannot be filter sterilized. Growth in the trypsin may not be seen because of its turbidity. Dou-

ble-check the trypsin source, dilute, and streak appropriate media with the trypsin to determine whether or not it is contaminated. It may be appropriate to check for mycoplasma. Trypsin is derived from pig pancreas and can be contaminated with low levels of swine mycoplasma.

Editorial Note:

Judy's points are well taken. I once read that the best managers are those who follow the principle of "management by wandering around." Perhaps that applies to biosafety professionals as well. Consider wandering into laboratories where biological safety cabinets are being used, evaluate the work practices, make constructive suggestions, and take the opportunity to do some "on the spot" training. It just might help the researchers and improve your relationships with them at the same time.

Errata

The Announcement "New NRM Registrants 2001" that appeared in *Applied Biosafety* (Volume 6, Number 3, 2001) on page 143 was incorrect. Robert P. Ellis, PhD, a new 2001 NRM Registrant, was mistakenly listed as being affiliated with South Dakota State University; rather, Dr. Ellis is affiliated with Colorado State University in Fort Collins, Colorado.