



Ask the Experts

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Do you have a biosafety question and you're not sure who to ask? Send your questions to the "Ask the Experts" column and I'll get them answered for you. Drawing from my own experience or that of other experts in the field, we'll try to compile a thorough and comprehensive answer to your question. Please e-mail your questions to jkeene@biohaztec.com or Co-Editor Barbara Johnson at barbara_johnson@verizon.net or Co-Editor Karen B. Byers at karen_byers@dfci.harvard.edu.

Biosafety Cabinets and Medical Waste

Question

We have three biological safety cabinets. All are class II, one is type A; the other two type B. All three passed their annual certification. In our laboratory, we use *Standard Methods 21st Edition* as a reference for monthly QC Equipment Checks. In 9020B, part m it states: "Once per month, expose plate count agar plates to air flow for 1 hr. Incubate plates at 35 degrees C for 24 hours and examine for contamination. A properly operating safety cabinet should produce no growth on the plates." Instead of one plate, we place 3 plates in the cabinet, one at each end and one in the middle. The last 3 months, we have seen one colony on different plates in different cabinets. We have repeated the test several times with the same results. The same sample plate does not grow a colony every time it is exposed. Finally, this month all plates have shown no growth. Could you provide some guidance on interpreting these results?

Response

Biosafety cabinet performance analysis is done in accordance with the National Sanitation Foundation (NSF) Standard 49. In this standard, there is a requirement that all biosafety cabinets pass a Product Protection Test. The test involves generating an aerosol containing 10^8 /ml spores positioned at the level of the work surface, in the center of the cabinet, and 4 inches outside of the work opening. The work surface is covered with open plates and the test is run for 5 minutes, after which the plates are covered and incubated. Under these very harsh conditions, a test is considered acceptable if the number

of CFU's within the cabinet does not exceed 5 CFU per test.

While "Standard Methods" states that "a properly operating safety cabinet should produce no growth on the plates," the universally accepted standard for biosafety cabinetry is the NSF 49 standard. This standard requires that under the test conditions, which should be done by certified testing personnel, the acceptable level must be less than 5 CFUs per test. Having said this, placing a single plate inside a cabinet and leaving it there for an hour should show no growth. However, because you are looking at random events, the test could show a positive result and the cabinet could still be functioning properly. You have placed three plates in the cabinet and that would increase the probability of capturing the random particles that might enter the cabinet, thus giving you a positive result.

A number of factors could affect the results of your testing. These would include personnel movement within the laboratory, operation of equipment that could affect airflow in front of the cabinet during the test, fluctuation in the HVAC system in the laboratory, personnel entering or exiting the laboratory at the time of the test and others. Because your "failures" are not consistent and not associated with either a particular cabinet or location within a cabinet, it does not seem probable that the cabinets are at fault, but rather random circumstances within the laboratory may be the problem.

Generally, if a BSC is properly certified on an annual basis, by a certified certifier, then the cabinet is working properly and any contamination occurring inside the cabinet is most likely due to a failure of the lab personnel to work properly within the cabinet. While doing lab safety and biosafety training, I have consistently found that only about 10% of the people who have BSCs in their lab actually know how the cabinets work. This lack of knowledge continues in spite of many excellent sources of information on BSCs and the continued effort of Biosafety Professionals to teach good practice.

Question

Our Laboratory SOP on Biohazards defines a biohazardous waste as follows: "a waste is considered to be infectious, and thus a biohazard, when it contains pathogens

of sufficient virulence, and in sufficient quantities that an infection may occur when a susceptible host is exposed to the waste.” Because of this definition, all samples from hospitals, medical laboratories, clinics and offices, diagnostic laboratories, animal experimentation units, research laboratories and dental offices are classified as biohazards and must be handled per the SOP using proper PPE. Years ago it was “determined” that any sample from the above locations that had a pH of <2 or >12 would not be considered a biohazard because of the extremes of pH. I was told that EPA, OSHA or CDC had provided the information. I have not been able to find any documentation.

Response

EPA defines medical waste as follows: “Medical waste is generally defined under state regulations. Medical waste is often described as any solid waste that is generated in the diagnosis, treatment, or immunization of human beings or animals, in research pertaining thereto, or in the production or testing of biologicals, including *but not limited to*:

- blood-soaked bandages
- culture dishes and other glassware
- discarded surgical gloves—after surgery
- discarded surgical instruments—scalpels
- needles—used to give shots or draw blood
- cultures, stocks, swabs used to inoculate cultures
- removed body organs—tonsils, appendices, limbs, etc.
- lancets—the little blades the doctor pricks your finger with to get a drop of blood.”

In addition, EPA gathered information during the two-year period of the Medical Waste Tracking Act and concluded that, “the disease-causing potential of medical waste is greatest at the point of generation and naturally tapers off after that point. Thus, risk to the general public of disease caused by exposure to medical waste is likely to be much lower than risk for the occupationally exposed individual.”

Currently, Medical Waste handling, transportation, and disposal are not regulated by the USEPA, but may be regulated on a “state-by-state” basis. Each facility should first determine whether or not there is a state regulation that applies and then follow that regulation.

If you are using the definition stated above and are considering **all** materials from those locations as biohazardous, then you are probably going too far. Note the definition requires two things: 1) the presence of pathogens of sufficient virulence, and 2) sufficient numbers of

those pathogens. These criteria are not always met by all specimens or materials collected in the noted locations. The determination of which wastes meet the definition requires a realistic evaluation by the generator. Laboratory personnel should always evaluate the materials with which they are working and, using reasonable criteria, differentiate between what is regulated and what is not.

You do not state what your proscribed PPE is for work with the medical waste, but again I would suggest that you evaluate the situation and your procedures in order to allow for reasonable interpretation and appropriate protection without undue burden on your personnel. Not all specimens from the above-mentioned locations are biohazardous and those that may contain infectious agents in small numbers, while potentially hazardous, can be handled differently than the cultures from those same specimens, which by virtue of their growth, would contain high numbers of organisms. As stated above by the EPA, the potential risk of exposure to infectious agents in Medical/Regulated Waste is far greater at the point of generation than once it has left the facility.

With regard to the pH issue, I can find no reference in either the CDC, EPA or OSHA documents on Regulated Waste that specifically state that materials potentially containing pathogens and had a pH of <2 or >12 would not be considered a biohazard because of the extremes of pH. (If someone reading this has found such documentation, please share it with us all.) Many factors could contribute to the survival of pathogens at either extreme acid or basic conditions. One thing we can say is that it is unlikely most human or animal pathogens would be viable in materials at either a pH <2 or pH >12. However, it is possible for them to be present in such materials. We do know that some intestinal organisms, under specific conditions, can survive at very low pHs. Therefore, if you are asked to look for infectious agents in materials at extreme pH ranges and someone thinks they might be present, the material should be treated as potentially biohazardous until it is shown not to be biohazardous.

References

National Sanitary Foundation International NSF/ANSI 49-2004a, NSF 49 Class II (Laminar Flow) Biosafety Cabinetry. November 11, 2004.

U.S. Environmental Protection Agency web site at www.epa.gov