Ask the Experts—Biosafety Requirements for Human Cell Lines

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Do you have a biosafety question and you’re not sure whom 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 to the Editor, Ira F. Salkin, at irasalkin@aol.com.

Biosafety Requirements for Human Cell Lines

As I was contemplating my article for this issue of Applied Biosafety, the topic of biosafety requirements for “human cell lines” popped up on the Biosafety Discussion List. It seems that we biosafety professionals have not done a very good job, in some instances, of explaining the differences between Biosafety Level 1 and Biosafety Level 2 practices to our researchers, and it is very important that we provide this most basic level of instruction. I hear over and over that a particular researcher feels that the imposition of Biosafety Level 2 practices is somehow going to interfere with his or her work and slow down or inhibit being able to perform research in an efficient manner. We often attempt to blame the Occupational Safety and Health Administration (OSHA) or some other regulating body for the requirements, and then the researchers comment that the requirement is “stupid” or has no “scientific basis.” It seems to me that we ought to put this problem to rest once and for all.

First, with regard to the inclusion of “human cell lines” under the Bloodborne Pathogens Standard (BPS), OSHA provided the following interpretation to Dr. Diane Fleming, ABSA President, on June 21, 1994. The letter is available on the OSHA web site at: (http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=INTERPRETATIONS&p_id=21519).

Dear Dr. Fleming:

This is in response to a September 23, 1993 letter from Joseph H. Coggin, an American Biological Safety Association member, requesting clarification of our August 3, 1993 letter of interpretation to the former ABSA President Dr. Jerome P. Schmidt. That letter attempted to explain the applicability of the Occupational Safety and Health Administration’s (OSHA) standard 29 CFR 1910.1030, “Occupational Exposure to Bloodborne Pathogens,” to establish human cell lines.

Dr. Coggin informed us that our August 3, 1993 letter may be more confusing rather than enlightening to biological safety professionals.

We have reconsidered our earlier comments and are providing a more detailed letter of interpretation. We regret any misunderstanding our earlier response may have caused.

As you know, the Bloodborne Pathogens standard (BPS) provides protection to employees who have occupational exposure to human blood or other potentially infectious materials (OPIM). Established human cell lines* (see attachment) which are characterized** (see attachment) to be free of contamination from human hepatitis viruses, human immunodeficiency viruses, and other recognized
bloodborne pathogens, are not considered to be OPIM and are not covered by BPS. Established human or other animal cell lines which are known to be or likely infected/contaminated with human microbes or agents classed as bloodborne pathogens, especially hepatitis viruses and human immunodeficiency viruses are covered by the BPS. The final judgment for making the determination that human or other animal cell lines in culture are free of bloodborne pathogens must be made by a Biosafety Professional or other qualified scientist with the background and experience to review such potential contamination and risk, in accordance with the requirements of the BPS. Documentation that such cell lines are not OPIM should be a matter of written record and on file with the employer for OSHA review.

All primary human cell explants from tissues and subsequent in vitro passages of human tissue explant cultures (human cell “strains”*, see attachment) must be regarded as containing potential bloodborne pathogens and should be handled in accordance with the BPS. Non-transformed, human cell “strains,” characterized by documented, reasonable laboratory testing as described in the attachment, to be free of human immunodeficiency virus, hepatitis viruses, or other bloodborne pathogens may be exempted from the standard’s requirements. However, if such tissue explants or subsequent cultures are derived from human subjects known to carry bloodborne pathogens, such as hepatitis viruses or human immunodeficiency viruses or are deliberately infected with bloodborne pathogens, they must be handled in accordance with the precautions noted in the BPS. Likewise, animal tissues, explants or cell cultures known to be contaminated by deliberate infection with human immunodeficiency virus or Hepatitis B virus are also subject to the BPS.

All laboratory work with primary human tissues or body fluids is covered by the BPS.

We hope this information is responsive to your concerns and thank you for your interest in worker safety and health.

Sincerely,

Ruth E. McCully, Director
Office of Health Compliance Assistance

Definitions

*A Human Cell LINE is defined as in vitro or animal passaged (e.g., nude mouse) cultures or human cells that fulfill traditional requirements of a cell line designation. That is, the cells are immortalized cells, transformed by spontaneous mutation or natural or laboratory infection with an immortalizing agent such as Epstein-Barr virus (EBV). EBV is a bloodborne pathogen. It should be noted that human cervical carcinoma cells or other transformed human cell lines like HeLa cells are sometimes adulterated with laboratory pathogens accidently introduced by cultivation with other cell cultures, or physically contaminated by other cell cultures handled in the same lab. In order to handle human HeLa cells, without having to comply with the requirements of the bloodborne pathogens standard (BPS), human HeLa cells should be documented to be pure HeLa cells and shown to be free of bloodborne pathogens by testing.

**Characterization of human cells, for inclusion or exclusion from compliance with the BPS, would include screening of the cells lines or “strains” for viruses characterized as bloodborne pathogens by the Standard, including human immunodeficiency viruses, hepatitis viruses or EBV, if the cells are capable of propagating such viruses. Most cell lines are screened for human mycoplasmas and are free of bacterial and mycotic contaminants. Testing may include antigenic screening for viral or agent markers, co-cultivation with various indicator cells that allow contaminants to grow, or using molecular technology (polymerase chain reaction or nucleic acid hybridization) to identify latent viruses capable of infecting humans such as Herpesviruses (e.g., EBV), or papilloma members of the Papovavirus group, etc. Cell lines that are procured from commercial vendors or other sources with documented testing to be free of human bloodborne pathogens and which have been protected by the employer from environmental contamination may be excluded from the BPS.

***Human cell STRAINS are defined as cells propagated in vitro from primary explants of human tissue or body fluids which have finite lifetime (non-transformed) in tissue culture for 20-70 passages. Human cell “strains” must be handled as potential biohazards unless characterized by testing to be free of bloodborne pathogens (i.e., WI-38 cells are often so documented).

This is pretty straightforward and is now available for all to read and keep on your desk to answer
any questions from your researchers. There is no question of "stupidity" or "scientific validity"; this is the interpretation of the law and failure to follow the law can result in serious consequences to the institution.

A second source of information on human cell lines that should be considered is provided by the American Type Culture Collection (ATCC) in their web site’s Frequently Asked Questions section (http://www.atcc.org/TechnicalInfo/faqCellBiology.cfm?q53). This resource is reprinted with permission from the American Type Culture Collection.

"Are ATCC human cell lines tested for viruses such as Epstein-Barr (EBV) virus, human immunodeficiency virus (HIV, AIDS virus), human T cell leukemia (HTLV), and hepatitis B virus? Are ATCC cell lines tested for bovine viral diarrhea virus (BVDV)?"

Answer: Some of our human cell lines are known to produce EBV, HTLV, or hepatitis virus, and this information is given in the catalog description and on product sheets. In addition, the human lung cell lines in our CCL collection have been screened and found negative for viruses by procedures that are detailed in our quality control manual (egg inoculation, hemadsorption, and co-cultivation with indicator cells). At this time, ATCC is distributing the HIV-positive line H9/HTLV-IIIB (ATCC CRL-8543). However, some of our other patent deposits have been derived from AIDS patients and may carry HIV.

Since it is not possible for us to test every cell line for every possible virus, we rely on the tests performed by the depositor. We recommend that all human cell lines be accorded the same level of Biosafety consideration as a line known to carry HIV." (Underline added by the author for emphasis.) "With infectious virus assays or viral antigen assays, even a negative test result may leave open the possible existence of a latent viral genome. Thus, it is best to use caution when handling any human cell line. Concerning BVDV, the virus is present in most serum samples, often at very low levels. Hence, it is probably present in all cell lines in which it can replicate unless the cultures have been grown in rigidly tested sera or sera of non-bovine origins. A paper describing testing of some ATCC lines was published in 1994 [S. R. Bolin et al., (1994) Survey of cell lines in the American Type Culture Collection for bovine viral diarrhea virus. J. Virol. Methods 48, 211]. Lines that are positive for BVDV are so described in the ATCC catalog descriptions."

Here it is very clearly stated that it is impossible to prove the negative. How do you know a pathogen is not present when you might not know that the pathogen even exists, until it shows up as a causative agent of a new disease? If you think this can’t happen, consider the HIV epidemic.

If we assume that the law requires us to prove that a material does not have infectious agents in it and to follow Biosafety Level (BSL)-2 procedures in a BSL-2 laboratory, how do we convince our reluctant researchers that we are not causing them to do something that will interfere in some way with their research? Look at the requirements for BSL-2 procedures and tell me if there is anything in these procedures that we really should not be doing. Following these Good Microbiological Procedures is important for the protection of the research as well as the personnel. These are the procedures, which are used in every clinical microbiological laboratory in the country and elsewhere. One does not have to have a PhD in microbiology to easily and efficiently follow these requirements. In fact, following them is good microbiological research. I encourage each biosafety professional to respond to the researchers who are concerned about the "hardships" of BSL-2 requirements by pointing out that the researcher is probably already following most of the BSL-2 requirements in a laboratory that would qualify as a BSL-2 laboratory.

It is the Law. Help them to understand that with little extra exertion, they can be compliant and reduce the potential for contamination of their research and themselves.