Standard Interpretations

06/21/1994 - Applicability of 1910.1030 to established human cell lines.

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• **Standard Number:** 1910.1030

June 21, 1994

Dr. Diane Fleming
President
University of South Alabama
College of Medicine
CSAB 170
Mobile, Alabama 36688

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 judgement for making the determination that human or other animal cell lines in culture are free of bloodborne pathogens must be made by a Bio-safety 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

Enclosure

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 immortalizating 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 accidentally 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).

September 23, 1993

Dr. Roger A. Clark, Director
Directorate of Compliance Programs
Occupational Safety and Health Administration
Washington, DC 20210

Dear Dr. Clark:

The American Biological Safety Association [ABSA], of which I am a member, recently contacted your office concerning the inclusion of "well established human cell lines" under the OSHA 29 CFR 1910.1030. I have a copy of your response letter dated August 3, 1993 to Dr. Jerome Schmidt, President of ABSA. Dr. Schmidt had submitted the inquiry letter at the request of ABSA's Technical Review Committee. ABSA was seeking exclusion for the use of **well characterized** human cells lines from the Standard when the lines have been proven virus/agent free by rigorous techniques. Dr. Schmidt's letter to you of March 25, 1993 acknowledges that "primary cultures" of human cells are potentially risky and require Universal Precautions. Well characterized human cells referenced in the ABSA inquiry means, I believe, **transformed lines** of human cells that have been tested with rigorous methods [e.g., culture, viral or agent antigen or markers, PCR in the case of human lymphocytes or epithelial cells for HIV or HBV, respectively].

Two statements in your response cause me grave concerns as a biological safety professional. First, your statements go much further than ABSA members ever expected when you included, by implication, that "protected" established cell lines, "primary cell lines" [Strains?] as well as secondary or higher passaged human cells were excluded from the Standard. According to your letter, cell strains cultured from primary explants or subcultures after passage 1 would not be covered by the BBP Standard. Most virologists recognize that many such human subcultures of primary cells, endogenously infected in the donor with silent HTLV viruses, papilloma, JC, BK, CJ, herpes, hepatitis and other viruses, as well as possible intracellular bacterial pathogens may represent a real and present source for human infection. A person receiving secondary or subsequent cultures of human lymphocytes, fetal cell mixtures, or hepatocytes from a vendor or laboratory may be obtaining human cells that contain a myriad of human viruses including hepatitis viruses and even HIV without any knowledge that the agents are present. Recall that 1 in every 250 American donors of tissue today may have HIV and that many more persons may harbor HBV. Such human cell "strains" would not require careful testing to determine their status as infectious agent free cultures so long as they are not "primary cultures" or deliberately infected with HIV. According to your recommendation, these passages of cells can now be handled by personnel

without compliance with 29 CFR 1919.1030. Rest assured, if this door is left open, many will use your statement in this way, even though I do not believe that is what you and OSHA meant to happen. **All human cell primary explants, derived cell strains from these explants, at any passage, and established human cell lines should be included under the standard unless well characterized by rigorous techniques and shown to be free of the BBP agents.**

The second statement of concern in your letter is that "Established cell lines, which are protected from contamination with environmental organisms to ensure their integrity for research purposes, are not considered OPIM and, are therefore not covered under the Bloodborne Pathogen Standard". You then clarify this statement implying that if HIV is [deliberately] cultured in the cells, the established cell lines are included under the Standard. It is my considered opinion that your official interpretation will now cause great confusion. Human cell lines from the American Type Culture Collection [ATCC] and other sources bear clear warning that they may contain BBP. ATCC recommends that these cells must be handled at BL-2 and in compliance with the BBP Standard. It is clear that some BBPs, especially endogenous human retroviruses can be harbored in **established cells**. If taken literally, your statement says that these cells may be considered excluded from the BBP Standard as long as they are kept **protected from contamination in the laboratory** handling them. In fact, they may already be contaminated with a spectrum of viruses, some of which can only be detected with nucleic acid blotting techniques that are not used routinely to screen for common viruses. So long as the receiving lab protects them from contamination with environmental pathogens in that lab, handling them does not require compliance with the BBP Standard. This is a potentially dangerous precedent that will almost surely lead to a laboratory exposure to BBP in the American work place. Such established cells showing no active viral replication, may be induced by a variety of agents to replicate endogenous viruses that are capable of infecting humans, especially if a worker is cut handling the cultures. I know you meant to be helpful in making the statement; however, many lab workers and especially their supervisors are more interested in getting around having to comply with the Standard than in seriously considering the true risk. They will contend that they did not expose the cells to environmental pathogens in their handling and this may be true, but not relevant, if the cultures are already contaminated upon receipt in the lab. Many labs do not have knowledgeable biosafety professionals with real expertise to correctly advise them about the requirements for characterization of established cell lines to reasonably establish the lines are likely to be viral or agent free. Now these labs will have license to do so without fear of regulation so long as they do not culture the cells with other cultures of BBPs.

ABSA was only asking for permission to exclude only **well characterized** human cell lines. Your letter gives authorization to **exclude** any human cell line, including secondary explants, so long as it is protected from environmental contamination with BBP in the recipient laboratory. Again, the cell line may already harbor BBP when received, but ignorance in this case would be adequate excuse to avoid compliance with the BBP Standard.

Please reconsider these two statements in your letter very carefully. I support ABSA's request for excluding rigorously characterized human cell lines, proven to contain no BBPs by stringent techniques [PCR, sensitive antigen detection, stimulation and co-culture assays, enzyme analysis, etc], but the wording of your letter will generate great confusion when I know that you were attempting to be helpful and cooperative.

Sincerely yours,

Joseph H. Coggin, Jr. Ph.D.
Professor and Chair, Microbiology and
Immunology, Professor of Pathology, and Associate Dean

November 10, 1993

Dr. Jessica Sandler
OSHA
Office of Compliance Programs
Occupational Safety and Health Administration
Washington, DC 20210

Dear Dr Sandler:

Thank you for your phone call regarding my letter of September 23, 1993 to Dr. Roger Clark, Director of The Directorate of Compliance Programs of OSHA. A copy of his response to Dr. Schmidt of ABSA is enclosed for your reference, along with a suggested redraft that I composed to deal with the issues of concern raised in my letter to Dr. Clark. As you can see I kept to the theme of his letter, but believe I used more traditionally accepted definitions of terms used to refer to tissue cultures.

I hope that these changes will be specific enough to be clarifying and faithful to the classic, widely accepted definitions of the terms "cell line" and "cell strain". The draft I enclose, hopefully will avoid the confusion I noted in the letter from Dr. Clark. I also defined the term "Characterization" to provide employers with a clear indication of the general laboratory testing criteria which should be used to establish human cell lines and strains as safe from the most problematic, non-treatable human blood borne pathogens.

Thank you for this opportunity to be of service.

Sincerely,

Joseph H. Coggin, Jr. Ph.D.
Professor and Chair and Professor of Pathology

August 3, 1993

Mr. Jerome P. Schmidt
President
American Biological Safety Association
1202 Allanson Road

Mundelein, IL 60060

Dear Mr. Schmidt:

This is in response to your letter of March 25, requesting an interpretation of the Occupational Safety and Health Administration (OSHA) standard 29 CFR 1910.1030, "Occupational Exposure to Bloodborne Pathogens." Specifically, you requested information as to the applicability of established human cell lines to the bloodborne pathogens standard.

As you know, the standard provides protections to employees who have occupational exposure to blood or other potentially infectious materials (OPIM). Established cell lines, which are protected from contamination with environmental organisms to ensure their integrity for research purposed, are not considered to be OPIM, and are therefore not covered under the bloodborne pathogens standard. However, please bear in mind that established cell lines containing the human immunodeficiency virus (HIV) are covered by the standard.

Primary cell lines, except those containing HIV, are also not covered by the standard. However, employees who initially handle the tissue from which any human cell lines are derived and do the initial steps in the culture of the cells are covered by the standard because of their reasonably anticipated exposure to unfixed tissues and blood.

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

Sincerely,

Roger A. Clark, Director
Directorate of Compliance Programs