What’s new, what’s hot, what’s timely? If you don’t have time to search the Internet for the latest developments that might impact your work environment, you just might find some of this information in this “Capsule” column. Please e-mail any comments or suggestions to felix.gmuender@bh.com.sg or to Co-Editor Barbara Johnson at barbara_johnson@verizon.net or Co-Editor Karen B. Byers at karen_byers@dfci.harvard.edu.


Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories

Prevention of injuries and occupational infections in U.S. laboratories has been a concern for many years. The Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH) addressed the topic in their publication Biosafety in Microbiological and Biomedical Laboratories (BMBL), now in its fifth edition (U.S. Department of Health and Human Services, 2009). However, BMBL was not designed to address the day-to-day operations of diagnostic laboratories in human and animal medicine. In 2008, CDC convened a Blue Ribbon Panel of laboratory representatives from a variety of agencies, laboratory organizations, and facilities to review laboratory biosafety in diagnostic laboratories. The members of this panel recommended that biosafety guidelines be developed to address the unique operational needs of the diagnostic laboratory community and that they be science-based and made available broadly (Miller et al., 2012). These guidelines promote a culture of safety and include recommendations that supplement BMBL by addressing the unique needs of the diagnostic laboratory. They are not requirements but recommendations that represent current science and sound judgment that can foster a safe working environment for all laborators. Throughout these guidelines, quality laboratory science is reinforced by a common-sense approach to biosafety in day-to-day activities. Because many of the same diagnostic techniques are used in human and animal diagnostic laboratories, the text is presented with this in mind. All functions of the human and animal diagnostic laboratory—microbiology, chemistry, hematology, and pathology with autopsy and necropsy guidance—are addressed. A specific section for veterinary diagnostic laboratories addresses the veterinary issues not shared by other human laboratory departments. Recommendations for all laboratories include use of Class II A2 biological safety cabinets that are inspected annually; frequent hand washing; use of appropriate disinfectants, including 1:10 dilutions of household bleach; dependence on risk assessments for many activities; development of written safety protocols that address the risks of chemicals in the laboratory; the need for negative airflow into the laboratory; areas of the laboratory in which use of gloves is optional or is recommended; and the national need for a central site for surveillance and nonpunitive reporting of laboratory incidents/exposures, injuries, and infections.


A Method for Evaluating Health Care Workers’ Personal Protective Equipment Technique

Beam et al. (2011) aimed at investigating the behavior of health care workers while using personal protective equipment (PPE). The proper usage of PPE and compliance with the procedures form an important line of defense to protect the patient and the health care worker. Because a bedside study would raise both ethical and legal concerns, the authors designed a study in a simulated patient care environment. The encounters between health care worker and patient were video-recorded. A fluorescent marker was used as a measure of contamination. The participants included 10 registered nurses, respiratory therapists, and nursing assistants from varying units of the hospital. Each participant had to carry out interventions on a patient based on his or her professional role. The authors note that although the number of participants was small, this allowed them to get information about the participants’ responses, which could be used in the design of future studies. The study participants were provided with a cart stocked with PPE (gowns, gloves, surgical masks, N95 respirators, and different kinds of protective eyewear). The fluorescent marker was applied at all places in the room where patient contamination typically occurs (bedrails, bedside table, and
the simulated patient’s gown front and arms). One half of the study participants could see a Centers for Disease Control and Prevention (CDC) poster on PPE donning and doffing in the room, but this study design had no detectable effect on their behavior. The participants were given verbal instructions on the patient scenario but no further instructions on PPE usage. The authors ensured that the fluorescent marker was dusted in the same way for each participant and cleaned if required (ultraviolet light monitoring). Each encounter was recorded with a high-quality digital video system. After each encounter with a patient, the participant removed the PPE and washed hands, before the remaining fluorescent marker on their body was digitally recorded (video, photography) and assessed by the same three reviewers. Beam et al. (2011) reported that the main breaches for PPE donning included not sealing the N95 respirator, not tying the gown at both the neck and waist, and donning the PPE in the wrong sequence. The most common mistake for PPE doffing was the wrong sequence and how contaminated items were removed from the room. During the encounter with the patient, the most common mistakes were touching unprotected areas of the study participant’s own body with contaminated PPE (gloves), and unnecessary touching of surfaces in the room during the interaction with the simulated patient. Eight participants had contaminated their body (6 on the hands, 3 on the back of their head, including one with a contamination at both places). Six participants had touched surfaces in the room that were unnecessary. Beam et al. (2011) conclude that the simulated patient care environment is effective to observe staff behavior and to evaluate the risk factors for non-compliance with PPE usage and protocols. Interestingly, they note that high-quality video recording and reviewing were more effective than the powdered fluorescent marker in observing non-compliant behavior. A portable high-quality video system could be used in actual health care settings as a valuable tool to extend the research beyond an educational laboratory setting. Camera positioning and repositioning and at the same time not cuing the participant (e.g., telling the participant to step back or stand in a certain way) is important for gaining valuable results.


Zoonotic Viruses Associated with Illegally Imported Wildlife Products

Smith et al. (2012) report that trade in live animals and animal products has led to the emergence of several zoonotic pathogens, including SARS, and illegal import of wildlife and wildlife products poses an increasing and substantial risk to public and agricultural health and native wildlife. Until recently, the health risks associated with legal and illegal wildlife import have gone widely unnoticed because of a general minimal surveillance of this trade, at least in the United States. Known examples of disease introduction to the U.S. include monkey pox, exotic Newcastle disease, and amphibian chytridiomycosis. As the case of monkey pox shows, a single shipment—whether legal or illegal—can result in a serious public health problem. In 2008 the authors (Smith et al., 2012) together with the Centers for Disease Control and Prevention (CDC) and inter-agency and non-governmental partners set out to assess the risks emanating from the illegal import of bushmeat. Harvesting and consumption of non-human primates (NHP) bushmeat has resulted in cross-species transmissions of several retroviruses to humans. Bushmeat confiscated at five U.S. airports was screened for multiple pathogen DNA including zoonotic viruses. The samples confiscated at the airports included 29 from NHP and 35 from rodents. Simian foamy virus (SFV, a simian retrovirus) was detected in 7 NHP bushmeat samples, herpesviruses (cytomegaloviruses and lymphocryptoviruses) in 11 samples, and both viruses in 4. The rodent samples were negative for leptospirosis, anthrax, herpesviruses, filoviruses, paramyxoviruses, coronaviruses, flaviviruses, and orthopoxviruses. The finding of SFV DNA in NHP samples is significant because SFV is a known zoonosis. The public health consequences of SFV are not fully understood because it probably cannot easily spread from human-to-human. Simian immunodeficiency virus (SIV) and simian T-lymphotropic virus (STLV) were not found in the small sample. Smith et al. (2012) quote other studies that found a high prevalence of these viruses at bushmeat markets. All the same, the authors (Smith et al., 2012) suggest that the results highlight a potential public health risk of exposure to legally or illegally imported wildlife and wildlife products that can only be assessed more thoroughly through broader surveillance and the discovery techniques tested in their study.


Scientists Rush to Find Clues on New Animal Virus

The new animal virus that was first detected last year in Germany and the Netherlands is spreading and taking a heavy toll on livestock across Europe, as reported in the News and Analysis section of Science (Kupferschmidt, 2012). The virus was termed Schmallenberg after the small town in Germany where it was first detected and from where it spread to the Netherlands, France, Belgium, the United Kingdom, Italy, and Luxembourg. Last summer, scientists at the Friedrich Loeffler Institute (FLI) in Germany first identified the virus in blood samples from cows that had shown signs of an infection. Symptoms are most serious in newborn sheep, goats, and cattle. The virus appears to have infected the fetuses of pregnant animals in summer 2011. By March 2012, more than 1,400 farms across Europe were affected. The virus belongs to the group of orthobunyaviruses that occurs mainly in Asia, Africa, and...
Australia. It is vector-transmitted by midges and mosquitoes. According to the FLI researchers, very little is known about this virus, but it appears not to be zoonotic. The genetic code indicates that it is related to three viruses that were isolated from cattle in Japan: Shamonda, Akabane, and Aino, which form a subgroup of orthobunyaviruses called Simbu serogroup. This is the first time that a virus of this group has been identified in Europe. European veterinary health institutes are scrambling to develop a good diagnostic test (the PCR assay can only detect an infection while the viral DNA is in the blood) and a fast-track vaccine (MacKenzie, 2012).


---

**Molecular Biosafety**

Margy S. Lambert

BIOSHERM, LLC, Madison, Wisconsin

The molecular biology and biotechnology fields are growing by leaps and bounds. Molecular Biosafety aims to shed light on how these cutting-edge techniques impact safety. Please e-mail your insights and questions to Margy Lambert at margylambert@gmail.com or Co-Editor Barbara Johnson at barbara.johnson@verizon.net or Co-Editor Karen B. Byers at karen_byers@dci.harvard.edu.

**Safety Considerations for Fusion Protein-Mediated Transduction Across Cell Membranes**

A number of proteins and peptides with therapeutic potential cannot readily cross cell membranes. A technique that fuses proteins, peptides, and oligonucleotides of interest to protein transduction domains (PTD) such as Trans-Activator of Transcription (TAT) overcomes this hurdle, bypassing the normal regulation of protein entry into mammalian cells (Yoshikawa et al., 2009). PTD fusion proteins are being used to effectively deliver biologically active macromolecules into a variety of cell types and for numerous *in vitro* and *in vivo* purposes including use as “protein therapy” for various diseases and disorders. The main safety questions that arise are: What are the potential hazards for researchers performing experiments using PTD fusion proteins as well as for subjects receiving PTD fusion protein therapy, and what precautions should be used to mitigate those hazards?

PTDs are short peptides (generally 10-20 amino acids) that are highly enriched in basic amino acids. The specific PTD motif can be tailored to maximize delivery of proteins into different cell types. A consecutive 11 amino acid arginine motif (11R), for example, enables entry into many cell types and is used effectively for delivery of proteins into neuronal cells (Ogawa et al., 2009). The TAT PTD sequence is derived from the human immunodeficiency virus (HIV) TAT gene, but the TAT PTD peptide presents no infectious risk.

While the entire TAT protein (consisting of 86 to 101 amino acids, depending on HIV subtype) is proinflammatory and can cause neurotoxicity when shed by HIV-infected cells (Chen et al., 2011), the TAT peptide (9 amino acids) that serves as a PTD has no associated toxicity. In fact, PTD peptides generally do not appear to show significant toxicity (Jones et al., 2005). Instead, hazards associated with PTD fusion proteins lie primarily with the effects of high levels of the specific protein in a particular cell type/tissue at a specific time/stage in the cell cycle.

Successful targets of protein therapy using PTD transduction include: 1) Increased expression of tumor suppressor proteins such as p53 in cancer cell lines and tumor tissues (Yan et al., 2012; Zhao et al., 2011a); 2) Increased expression of metallothionein and superoxide dismutase in neuronal cells for prevention of diabetes and diabetic neuropathy (Min et al., 2012; Park et al., 2011); 3) Increased expression of a peptide that prevents degradation of a transcription factor regulating expression of a number of cytoprotective genes in brain-injured tissues (Zhao et al., 2011b); and 4) Increased expression of a DNA repair protein to protect against radiation-induced damage in salivary glands (Sunavala-Dossabhoj et al., 2012).

The level of potential hazard associated with a PTD fusion protein is likely dependent on the peptide or protein attached to the PTD that is delivered into the cell. A biologically active protein or peptide with negative effects, such as an oncoprotein, poses a higher hazard than a housekeeping protein, while introduction of proteins demonstrating positive effects, such as tumor suppression in cancer cells, are actually beneficial when expressed in the right place, time, and subject.