

Transfer of Biological Samples from a Biosafety Level 3 Facility

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In the last two decades, since the 1990's, the increased concern about emerging and re-emerging diseases, mostly zoonotic bacteria or viruses (West Nile virus in USA, SARS corona virus and highly pathogenic avian influenza virus in Asia, the new H1N1 influenza worldwide) has fueled the design, construction and operation of a multitude of new biosafety- biocontainment facilities and/or laboratories [1-3] in USA, in Europe as well as in Asia and Africa. Both biosafety level 3 (BSL3) and biosafety level 4 (BSL4) facilities, which study human or animal pathogens, with specific difficulties, levels and labels, [4,5] are highly technical and complex in their construction, commissioning, and management [6]. The building costs for a BSL-3 facility are typically double or quadruple those of a BSL-2, with even greater differences evident when taking into consideration operational costs such as energy, maintenance, and dedicated personnel (from 200-800%). Such is the pressure to reduce the area devoted to BSL3-BSL4 activities that, occasionally, the final working spaces are so small as to force the transfer of samples to the outside. This fact could have serious implications for both biosafety and biosecurity issues, two different [7], yet closely related terms, as improperly treated and therefore still infectious materials transferred out of a BSL3/4 facility could lead to potential proliferation of bio-weapons and increase the biohazard to the community. A biological sample can reach the outside of a BSL3 area in two ways: either without inactivation (infectious) if it must be transferred to another BSL3 facility, or after undergoing an inactivation process to render the biological sample non-infectious.

Transferring infectious samples

Researchers commonly refer to specific guidelines (Laboratory biosafety manual from WHO, PHAC, BMBL or NIH-CDC, [8-10], which distinguish between Category A and B infectious substances [11] and their methods of shipping, but specific regulations within each country should also be considered. In this case the danger exists in the transportation of the biological specimen between facilities (bio security issues may also play an important role), but not necessarily in their further handling as those materials will be used, propagated and tested in an equivalent BSL3 facility.

Transferring non-infectious samples

This is an important issue with further human and livestock health implications as well as economical impacts if inactivation is not properly performed. In this way, we can distinguish two main types of inactivation procedures. Firstly, *in house* procedures, following internal protocols, many of which may have been previously reported in published papers, reviews, etc. in scientific journals. Several procedures have been used for many years with seemingly good safety records (although difficult to quantify in terms of log₁₀ reduction of viral infectious titers). These include thermal procedures, inactivation by solvent or detergents [12], use of chaotropic agents, phenol: chloroform extraction procedures, desiccation on specially-treated papers (FTA cards), as well as inactivation by fixation for anatomical pathology purposes [13-16]. Secondly, in many cases the inactivation procedure relies on the capacity of the initial steps of current commercial nucleic

acid extraction kits which many researchers and lab technicians consider as viral or bacterial commercial inactivation kits [17]. The choice of inactivation treatment (or step) should be closely related to the final destination of the sample (anatomical pathology, molecular biology, immunological techniques, biochemistry, etc.). In molecular biology techniques, initial steps usually include the mixture of biological samples with lysis buffers (containing chaotropic agents at unknown concentrations), in addition to further thermal steps in some cases. BSL3 facility management staff commonly regards biological materials mixed with lysis buffers as non-infectious. However, manufacturers do not provide any evidence of such effects in any product data sheets, and there is limited data in the journals. In fact, it is difficult to compare the inactivation capacity of lysis buffers because some claim to contain guanidine derivatives without specifying the amount. In many cases, there is neither proper documentation of the exact compounds included nor their concentrations. In our facility, lysis buffers containing guanidine have shown very disparate inactivation rates for a specific virus, making it impossible to draw general conclusions about all lysis buffers. A case-by-case validation for each buffer should be performed, in order to accept a safe transfer of the treated samples to the outside. Indeed, the validation of these nucleic acid extraction kits should also be mandatory for BSL3/4 facilities. If we accept that a lysis buffer is acceptable for inactivation of a virus handled in a BSL3/4 environment, without previous *in house* testing, and therefore allow those mixtures to be further handled on the bench of a BSL3 facility, or even within a BSL2 environment, we may compromise the health of our lab workers, increasing the risk of laboratory acquired infections (LAI) within our facility. Cases of laboratory worker infections from improperly inactivated or mishandled biological samples have been previously reported [18,19]. There are recent examples of LAI by SARS (20), tularemia infection among workers in Boston University, Q fever and *Brucella* infection in Texas University workers, virulent H5N1 mistakenly mixed with H3N2 in a European lab, etc.

Finally, most of the materials undergoing inactivation are not regarded as dangerous by the recipients. Although the laboratory releasing the samples should keep a record of the inactivated materials exiting its facility, this record is often lost when the material arrives at the recipient institute.

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Need to perform validation tests and standardize and share data

There is no clear and simple way to perform viral validation studies in biocontainment facilities with BSL3 pathogens. The establishment of certain guidelines for viral inactivation with threshold standards of efficacy ($>4 \log_{10}R$), and the creation of a bank of potential viruses to be tested, would be highly advisable. When testing commercial nucleic acid extraction steps, minimum criteria (worst cases in reagent concentration, temperature or contact-times) should be determined in order to fully challenge the robustness of the step or reagent. It should also be mandatory to test the viruses actually in use within the facility. A general data-base comprising the capacity of all commercial lysis buffers, thermal treatments, as well as *in house* inactivation methods used at each institute or facility should be set up in order to share the viral or bacterial inactivation data generated from each validation test, and to ultimately save money and invest in common inactivation strategies. In overview, the removal of inactivated samples from a BSL3 facility must be limited at a minimum, and avoided entirely whenever possible. Although the BSL3/4 environment is highly expensive, it is necessary to find ways to perform the majority of activities with BSL3 pathogens inside a BSL3/4 facility. Clearly, the higher the number of inactivated biological samples reaching the outside of BSL3 facilities, the more likely a potentially dangerous situation may arise.

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