Biosecurity and biosafety: Veterinary laboratories and animal facilities

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INTRODUCTION

Laboratories that handle pathogens (biological laboratories, see Definitions) have special requirements in terms of ensuring health and safety. The issues are protection of the personnel who work with the pathogens or at least in areas where the pathogens are handled or stored and protection of the environment and the public at large from contamination that could result from release of pathogens from the laboratory. The latter includes preventing the intentional release of pathogens to commit acts of bioterrorism. In the context of laboratories, these activities are given separate definitions, with the term ‘biosecurity’ sometimes reserved for the measures applied to prevent theft of pathogens with malicious intent to cause harm. However, the simplest concept is that biosafety refers to the measures in place to protect humans and the environment while biosecurity refers to the measures that protect the pathogens from damage and wrongful removal (accidental or intentional). A number of definitions as applied to laboratories that handle pathogens are given below.

It is recognised that in addition to biological hazards due to the presence of infectious material, biological laboratories can pose chemical and physical hazards as well, and therefore the biosafety recommendations include measures to protect personnel from those hazards and to ensure their safe use, storage, management and disposal.

Appropriate management of highly dangerous pathogens requires considerable investment in terms of finance as well as commitment and dedication on the part of personnel. The resources required might be beyond the reach of smaller facilities and a risk-based approach is recommended. The concept of the bio-risk approach is embodied in guidelines by the World Health Organization (WHO) published in 2006. The document highlights events that have influenced a change in the way that biosafety and biosecurity are implemented in biological laboratories. These include the infection of laboratory workers with SARS-CoV in 2003-2004 in Singapore, Taipei and Beijing and the need to respond to public and regulatory concerns with regard to the maintenance in research facilities of the agents of eradicated diseases (smallpox variola virus, rinderpest virus) and events involving the misuse of biological agents to cause harm, such as the 2001 anthrax letters in the USA. The document points out that more adequate provision for data access might have permitted more suspected institutions and persons to be eliminated from the investigation into the anthrax letters event.

Veterinary laboratories are under no less pressure or obligation to exercise adequate biosafety and biosecurity measures. It has been pointed out that a high percentage of emerging pathogens affecting humans are associated with animal reservoirs, so that material entering veterinary diagnostic laboratories must be regarded as posing a potential threat to human as well as animal health; of course, many of the known established pathogens of animals are also zoonotic. In providing standards and guidelines for biosafety and biosecurity in veterinary laboratories the OIE follows a risk-based approach to categorise pathogens according to level of hazard. According to WHO the global trend for managing laboratory biosafety and biosecurity is not prescriptive but involves goal-setting: performance expectations are described for facilities, which are expected to provide evidence of the appropriateness and validity of their risk minimization measures.

It should be noted that the chapter on laboratory biosafety and biosecurity of the OIE manual does not directly address the question of misuse of pathogens, although the prevention of this is implicit in the
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prevention of release of pathogens into the environment where they may cause disease in humans and animals. However, it is evident that a number of the pathogens found in veterinary laboratories are potential candidates for bioterrorism and that a lower level of biosecurity than that recommended for laboratories for human diseases could result in veterinary laboratories being targeted by people wishing to cause harm through the use of biological agents.

DEFINITIONS

The OIE Terrestrial Manual for Diagnostic Tests and Vaccines does not provide definitions for the terminology for biological laboratories in the Glossary, therefore the definitions used here are those provided by the World Health Organization in 2006.

**Biological laboratory:** A facility within which micro-organisms, their components or derivatives are collected, handled and/or stored.

**Biorisk:** The probability or chance that a particular adverse event, possibly leading to harm, will occur.

**Biorisk assessment:** The process to identify acceptable and unacceptable risks (embracing biosafety risks (risks of accidental infection) and laboratory biosecurity risks (risks of unauthorized access, loss, theft, misuse, diversion or intentional release)) and their potential consequences.

**Biorisk management:** The analysis of ways and development of strategies to minimize the likelihood of the occurrence of biorisks.

**Biosafety:** Laboratory biosafety describes the containment principles, technologies and practices that are implemented to prevent the unintentional exposure to pathogens and toxins, or their accidental release.

**Laboratory biosecurity:** Laboratory biosecurity describes the protection, control, and accountability for valuable biological materials within laboratories, in order to prevent their unauthorized access, loss, theft, misuse, diversion or intentional release.

**Valuable biological materials:** Biological materials that require (according to their owners, users, custodians, caretakers or regulators) administrative oversight, control, accountability, and specific protective and monitoring measures in laboratories to protect their economic and historical (archival) value, and/or the population from their potential to cause harm.

An additional term used is bio-containment, which is defined by Merriam-Webster Dictionary (http://www.merriam-webster.com/dictionary/biocontainment) as ‘the containment of extremely pathogenic organisms (as viruses) usually by isolation in secure facilities to prevent their accidental release especially during research’.
ASSESSING THE RISK POSED BY PATHOGENS IN A VETERINARY LABORATORY SETTING

Risk assessment is based on knowledge of the epidemiology and characteristics of the pathogen, in particular its infectivity for humans and animals, the routes of transmission, its stability/ability to persist in the environment and whether prophylactic measures and treatment are available. It also considers the consequences of infection of humans and animals with the pathogen. Using this approach, the OIE classifies pathogens into four groups, which in turn determine the measures that must be implemented to ensure their containment.

Recent decades have been characterized by the emergence of a number of ‘new’ pathogens of human and animals. When such pathogens appear little or nothing is known about them, and therefore it is not possible to undertake a risk assessment in the same way as for a known pathogen. In this case the risk assessment can be based on the information available about related pathogens if any, and in the absence of good information will use the precautionary principle, which assumes a ‘worst case scenario’. However, usually by the time samples are submitted to a laboratory for diagnosis they represent a clinical manifestation that will provide some idea of the severity of the disease and the proportion of an animal population that has been affected. The emergent pathogen will often be discovered through a process of elimination of known pathogens that cause similar phenomena, and will likely be considered to pose the same level of risk as those pathogens, although this might later be disproved. All samples presented to a veterinary laboratory should be handled with at least basic biosafety precautions not only to protect the handlers from infection but also to protect the sample from contamination that might result in a misdiagnosis or failure to make a diagnosis.

Apart from the inherent characteristics of the pathogen, there are other factors that influence the level of risk that must be taken into consideration in developing biosafety guidelines. These include the amount/volume and concentration of the pathogen or culture that will be handled and the procedures that will be carried out with it; for example producing antigen or vaccines exposes the handler to much larger quantities of the pathogen than testing or culturing it for diagnostic purposes. The state of health of those exposed must also be considered, as people with lower immunity including pregnant women will be at higher risk than other members of staff and may need to be excluded from handling certain pathogens. Pathogens that depend entirely upon arthropod vectors for transmission will pose a lower risk if the laboratory is situated in an area from which the vectors are absent. More details about some of the issues to consider when assessing the risk are provided in the OIE Terrestrial Manual.
OIE has grouped pathogens causing animal diseases into four groups based on risk assessment:

### OIE grouping of pathogens according to level of risk

<table>
<thead>
<tr>
<th>Group</th>
<th>Criteria (all veterinary pathogens)</th>
<th>Criteria (animal pathogens)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Unlikely to cause human or animal disease &lt;br&gt; Cause endemic animal diseases not subject to official control</td>
<td>Disease-producing organisms &lt;br&gt; Endemic &lt;br&gt; Not officially controlled</td>
</tr>
<tr>
<td>2</td>
<td>May cause animal or human disease &lt;br&gt; Unlikely to spread in human or animal populations &lt;br&gt; Effective prophylaxis and treatment are available</td>
<td>Disease-producing organisms &lt;br&gt; Exotic or endemic &lt;br&gt; Subject to official control &lt;br&gt; Low risk of spread from laboratory</td>
</tr>
<tr>
<td>3</td>
<td>Can cause severe animal or human disease &lt;br&gt; May spread in human and/or animal populations &lt;br&gt; Effective prophylaxis and treatment are usually available</td>
<td>Disease-producing organisms &lt;br&gt; Exotic or enzootic &lt;br&gt; Subject to official control &lt;br&gt; Moderate risk of spread from laboratory</td>
</tr>
<tr>
<td>4</td>
<td>Cause severe animal or human disease &lt;br&gt; May represent high risk of spread in human or animal populations &lt;br&gt; Usually no effective prophylaxis or treatment</td>
<td>Disease-producing organisms &lt;br&gt; Exotic or endemic &lt;br&gt; Subject to official control &lt;br&gt; High risk of spread from laboratory to environment/animal population</td>
</tr>
</tbody>
</table>

The level of bio-containment required for any given pathogen is determined by the group to which it is assigned according to the above-mentioned characteristics. However, it is accepted that many facilities that are not equipped to handle Group 3 and 4 pathogens may receive samples that contain such pathogens and this will only become known when diagnostic assays have been performed. There are therefore certain basic sanitary requirements for all facilities that handle diagnostic specimens to protect the safety of people who work with them and that of the environment. They are the following and are listed by the OIE as essential requirements for any laboratory work:

- Laboratory surfaces easy to clean, waterproof and resistant to chemicals; facilities exist for hand-washing and dealing with emergencies (spills, contamination of personnel) as indicated by the work done in the laboratory; established protocols for frequent cleaning and disinfection.
- Access restricted including for personnel using appropriate measures e.g. electronic access.
- Appropriate personal protective clothing and equipment available; must not be worn outside the laboratory.
- Laboratory doors must be kept closed when work is in progress; ventilation provided by air extraction; ventilation systems must be balanced if using biosafety cabinets.
- No food or drink including oral medication to be stored or consumed in laboratories.
- No smoking or application of cosmetics in the laboratory.
- No pipetting by mouth.
- Production of aerosols must be minimized.
- Emergency response plans must be developed and in place.
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- Used glassware and other contaminated material must be stored, handled, decontaminated and disposed of safely and appropriately according to existing protocols.
- No disposal of infected material in any manner other than that prescribed by laboratory protocol.
- Any accident or incident must be reported to the Safety Officer (or other responsible person).

These provisions, which have been slightly modified from the list provided by the OIE Manual to allow some flexibility in how the purposes will be achieved, are required for Group 1 pathogens but will allow safe handling of unknown specimens to the point at which they can be identified or referred to an appropriate facility. Progressively more rigorous requirements are necessary for pathogens of Groups 2 – 3. The OIE Manual provides an extensive table indicating these requirements in terms of structure and location, additional laboratory requirements, laboratory discipline and handling of specimens. Requirements additional to the basic requirements listed above and not applicable to basic laboratory facilities are summarised briefly in the table below, but reference to the OIE Manual is advised. Some of the requirements that would apply to any laboratory facility e.g. not situated next to a known fire hazard and protected from access by rodents and insects have been omitted.

### Additional requirements for Groups 2 – 4 pathogens

<table>
<thead>
<tr>
<th>Requirement</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated location advisable</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Workplace separated from other facilities</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Liquid effluent must be sterilized and monitored</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liquid effluent from steam sterilizers must be sterilized and monitored</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Isolated by airlock with continuous air flow</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Laboratory under negative pressure, differentials monitored</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Input and exhaust air to be filtered with HEPA or equivalent filters</td>
<td>X</td>
<td>X</td>
<td>X¹</td>
</tr>
<tr>
<td>HEPA filters verified regularly (usually annually)</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Mechanical air supply system with fail-safe system and alarm</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Sealable to permit fumigation</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Incinerator, pressure steam sterilizer or renderer available</td>
<td>X</td>
<td>X</td>
<td>X²</td>
</tr>
<tr>
<td>Class I or II biological safety cabinet available</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Class III biological safety cabinet available</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Cabinet HEPA filters verified regularly (usually annually)</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Direct access to autoclave/pressure steam sterilizer</td>
<td>X</td>
<td>X¹</td>
<td>X²</td>
</tr>
<tr>
<td>Specified pathogens stored in laboratory</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Double-ended dunk tank required</td>
<td>Preferable</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Full body shower and change of clothing required before exiting</td>
<td>Sometimes</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Safety Officer responsible for containment</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Staff specially trained and demonstrate necessary competences</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Warning notices for containment area (hazard and contact person)</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Emergency protocols posted in laboratory</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Laboratory must be lockable</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Packaging requirements must be advised prior to submission</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Incoming packages opened by trained staff in appropriately contained reception area</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Movement of pathogens between laboratories requires a license</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Standard Operating Procedures covering all areas available</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

¹Double on extract; ²On site; ³Must have double doors for access
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ACHIEVING THE REQUIRED LEVEL OF BIOSECURITY

The topic of laboratory biosecurity is extensively covered in various documents provided by WHO as well as regional bodies like the European Commission and national bodies like the United States Department of Agriculture (USDA), DEFRA and others listed at the end of this module. The main concepts are briefly summarised.

As indicated before, biosecurity in laboratories refers to the protection of valuable biological material from harm or loss. While the misuse of laboratory stocks of pathogens has given impetus to the initiatives for increasing laboratory biosecurity, this is not the only purpose for ensuring that valuable biological material is properly preserved and stored. Its conservation in the best possible state is necessary to ensure its availability for future generations of scientists and therefore laboratory biosecurity depends not only physical barriers that prevent the material from being tampered with but also the creation and maintenance of ideal storage conditions and a recording system that ensures that the material is properly identified and can be traced when needed. Laboratories where reference material is kept can be considered as archives of irreplaceable scientific material that is priceless and deserves the greatest possible investment of resources to keep it intact for future research. Stored biological materials can also be of immediate practical use in supporting decision-making for disease control. When post-weaning multi-systemic wasting syndrome was diagnosed in South African pigs for the first time, a retrospective study on sera from annual serological surveys indicated that infection with porcine circovirus-2 had been present in the majority of pig herds sampled for at least eight years, indicating that adding the syndrome to the list of controlled diseases would be unhelpful.

The announcement of the global eradication of smallpox in 1978 and rinderpest in 2011 raised questions about the wisdom of retaining any infective material from those diseases. The debate about whether such material should be kept and if so where continues, but it seems most likely that some material will be preserved in a small number of designated facilities that are able to maintain the highest level of biosecurity.

It is pointed out by “WHO” that laboratory biosecurity can be breached not only by human agencies (unintentionally or maliciously) but also as a result of natural disasters that cause structural damage to the facility, for example earthquakes and that acceptable bio-risk management plan should be developed.

Laboratory biosafety in general supports laboratory biosecurity, and many of its requirements such as an uninterrupted electricity supply (i.e. an automatically activated standby generator), restricted access, self-closing doors, separation of work areas and emergency plans, are also biosecurity requirements. The “WHO” Bio-risk document identifies two potential areas of conflict. The first is that restricted access should not exclude an emergency response by rescue personnel, and this needs to be provided for in such a way that biosecurity will not be breached. The second is that signage for bio-hazard areas usually identify the agents present. This may not be desirable in terms of biosecurity, and the signs may need to be restricted to indicating the biosafety level, responsible person and his/her emergency contact details.
LABORATORY ANIMAL FACILITIES

Biosafety in animal handling facilities that are part of laboratories is covered in the OIE chapter with laboratories, as the basic principles and their implementation are the same. Biosecurity is of great importance in facilities where live animals are kept, since escapes of animals infected with pathogens are a major hazard in terms of releasing pathogens into the environment. Although modern technology has to a great extent eliminated the need to use laboratory animals for diagnostic assays, they are still used for some tests as well as for other purposes such as production of antigen and antisera. The physical security of the facility is therefore of the utmost importance in preventing accidental escapes of laboratory animals, as well as a high level of training and commitment in the personnel who are responsible for the care of the animals as well as those who use them in their work. In recent decades there have been incidences of intentional release of laboratory animals by people or groups opposed to their use and this has increased the need for assuring a high level of security. Although not directly involved with biosecurity, it is also important that such facilities implement protocols to ensure the welfare of the animals at all times and that this is documented in a way that is credible and verifiable. (See the module on Animal Welfare).

TRANSPORT OF SAMPLES TO AND BETWEEN VETERINARY LABORATORIES

International regulations for the transport of infectious substances are provided by WHO in a document that is accessible via the internet and is regularly updated. A chapter on the transport of specimens of animal origin is provided in the OIE Terrestrial Manual and covers both how samples should be transported from the field to a veterinary laboratory and how specimens should be transported between laboratories within a country for reference and quality assurance purposes. Regulations for international transport of infectious and toxic substances are provided in Volume 1 of the United Nations document on Recommendations for the Transport of Dangerous Goods.

The UN document defines six categories of biological specimens (infectious substances, cultures, patient specimens, biological products, genetically modified organisms/micro-organisms, medical or clinical wastes), of which the first three are of most importance for laboratories, with biological products also being of interest to facilities that produce vaccines and other so-called biologicals for veterinary use.

Infectious substance are subdivided into Category A and B. Category A is for substances defined by the UN as those 'transported in a form that, when exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease in humans or animals'. The UN provides a list of Category A infectious substances i.e. pathogens capable of causing the above in humans, many only when in culture form, as well as cultures of animal pathogens that do not affect humans (the agents of African and classical swine fever, foot and mouth disease, velogenic Newcastle disease, contagious bovine pleuropneumonia, peste des petits ruminants, rinderpest, lumpy skin disease, sheep-and goatpox, swine vesicular disease and vesicular stomatitis). Additionally, any emergent pathogens are placed in Category A. Category B includes all infectious substances not satisfying the criteria for Category A. The UN document provides for exemptions for specimens that are regarded by experts as posing no danger to humans or animals if released, noting
that these should nevertheless be transported in a way that preserves their integrity and prevents leakage and spillage.

The minimum packaging requirements for the transport of any biological specimens are designed to prevent breakage and leakage and to ensure that they reach the laboratory in the best possible condition as well as that they do not contaminate the handlers or the environment *en route* to the laboratory. Three layers of packaging are necessary:

- **Primary inner receptacle containing the specimen:** watertight, leak-proof, preferable not breakable, and surrounded by sufficient absorbent material to retain the full amount of liquid in the primary receptacle; no sharps should be present in the package. There can be one or multiple primary inner receptacles each individually wrapped in absorbent material.
- **Secondary container:** durable, watertight, leak-proof container (sealed plastic bag, screw top plastic jar or can) large enough to contain the primary receptacle/s and sufficient absorbent material to surround them and absorb the full amount of liquid in the package.
- **Outer packaging:** the outer shipping packaging consists of a rigid container (sturdy cardboard box, rigid cooler) with sufficient cushioning and if necessary thermal insulating material.

Identification of specimens is of paramount importance. Detailed identification of and information about the specimen should be placed in a plastic envelope in the second packaging between the packaging and the outer wall of the container.

The international regulations for transporting Category A infectious substances are rigorous and include using only UN-approved packaging, marking of the package with the information that it contains an infectious substance that affects humans or that affects animals only and the UN code (UN2814 for the former and UN2900 for the latter), and that personnel packing, shipping or transporting Category A substances must be trained and certified according to international regulations. The great majority of pathogens listed by UN as Category A have the qualification ‘cultures only’ so that patient specimens that might contain Category A pathogens listed as ‘culture only’ can be transported as Category B substances. Since very few of the pathogens that are listed as Category A substances in any form (i.e. not culture only) cause disease in animals, the probability of their being sent as specimens to a veterinary laboratory is low, so for this reason the requirements are not covered in detail in the OIE Manual.

Most infectious specimens or material submitted to a veterinary laboratory other than cultures of highly pathogenic organisms are either Category B or exempt. Detailed instructions for packaging and transporting Category B infectious substances are provided in the OIE Manual for surface transport in country, as well as additional requirements for air and international transport. In addition to the triple packaging requirement, there are volume and weight restrictions, specifications for the quality and strength of the packaging and specific labelling requirements. These include the shipping name Biological Substance Category B in letters at least 6 mm high, a biohazard sign (see Power Point) and a diamond-shaped mark at least 50 mm x 50 mm with a border not less than 2 mm thick and the code UN3373 in text no less than 6 mm high, as well as the delivery address, the sender’s details and names and telephone numbers for both the sender and the recipient. There are no international requirements for documentation for surface transport of Category B substances but there may be national regulations. However, for air transport a waybill is also required. There are also regulations for refrigerants, and in particular if dry ice is used, itself a dangerous substance, there are many additional requirements and special shippers are available, as well as certain authorised shipping...
agents. For international transport of specimens there are requirements in terms of movement permits, which are usually supplied by the recipient laboratory.

Communication with the receiving laboratory is essential prior to shipping. All stages (packaging, transport and reception) should be undertaken by persons trained in the handling of biological samples. Direct transport to the laboratory is recommended. Couriers are most reliable and some are registered for and experienced in the international transport of infectious material.

REFERENCES


