BIOSECURITY IN THE LABORATORY

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Summary: The authors examine the relationship between the risk levels of pathogens and the minimal biosecurity measures required in diagnostic and veterinary research laboratories. This type of examination is necessary so that handling of these pathogens becomes neither a health risk for persons working in such laboratories nor a danger for either domestic or wild animal populations.

Furthermore, the importance of biological security and of laboratory techniques and protocols is described. Also emphasised are the main characteristics of biosecurity equipment used as a front-line barrier for the containment of pathogens.

Ten OIE Member Countries in the Americas responded to a survey on the security in their veterinary laboratories. This survey shows that there are very few laboratories which have been correctly designed and equipped to handle pathogens while maintaining high levels of biosecurity. The majority of laboratories do not fulfil the biosecurity conditions necessary to properly handle the pathogens with which they currently work.

As we reach the end of the century and the third millennium, human society is becoming increasingly concerned with achieving the highest possible quality of life, in the midst of populations suffering from extreme poverty, underdevelopment and marginalisation, contrasting strongly with the amazingly fast technological progress which is taking place in every field of learning.

This situation is becoming more and more obvious with the unexpected increase in globalisation of the economy, with the opening up of markets and the formation of economic groups which threaten to eliminate national borders. We are gradually approaching the vision of a "worldwide village», to which we must continue to adapt, advancing at a faster, but also steadier pace.

In this respect, the Office International des Epizooties (OIE) has successfully taken the lead in its own branch, guiding its Member Countries in the modernisation of their veterinary services so that they can meet the provisions of the World Trade Organization Agreement on the application of Sanitary and Phytosanitary Measures. Both a conceptual and structural re-organisation is necessary, and the first step in the process is the updating of legislation, standards and procedures.

If countries are to be competitive on the live animal and animal products markets, they need to prevent, control and eradicate disease and strengthen and optimise the organisation of health assistance in the field of livestock raising, giving full consideration to the conservation of both human health and the quality of the environment.

Careful thought must therefore be given to the situation of animal health diagnostic and investigation laboratories on the American continent.

Enormous progress has been made in the field of diagnosis and, consequently, in the control and eradication of those diseases which have the greatest affect on the productivity of livestock raising. However, if a detailed examination of the structure and infrastructure of our animal health laboratories to determine the biosecurity level, were to be carried out, the results would be surprising.

Unfortunately, it is difficult to give a meaningful, realistic overview of the situation as only ten out of the twenty-one OIE Members Countries in the Americas asked to complete the questionnaire actually sent responses.
In conclusion, it should be emphasised that this report is intended as a warning for those in charge of the Animal Health Services in the Americas, regarding the apparently inadequate level of biosecurity in laboratories and the possible increase in the health risk for both people and animals in these countries.

1. INTRODUCTION

There is a high probability of diseases spreading outside the laboratory when no basic infrastructures are provided or when the standards and protocols which contribute to maintaining a high level of biosecurity are not respected on an everyday basis.

There are frequent cases of contamination of laboratory staff working with extremely virulent pathogens, and the infection of communities located in the vicinity of these diagnostic and investigation centres. Various epizooties have originated from these centres.

In this context, the authors investigated the situation of the laboratories on the American continent, based on universally accepted criteria for measuring the capacity to reduce risk levels and on documents published by Canada (2, 6), Colombia (8), United States of America (3, 7) and by international organisms such as the Inter-American Institute for Cooperation on Agriculture (5), the OIE (9) and the World Health Organization (10).

In this article, a relationship is established between the organisms handled (risk groups) and the physical characteristics of the laboratories concerned (minimum biosecurity requirements and equipment).

An analysis has also been made of the capacity of containment of these laboratories, based on the risk groups, the minimum containment levels and the results of the survey.

An analysis of the survey results shows that laboratory staff and/or animals are at a high level of risk.

2. LEVEL OF RISKS

To classify laboratories in terms of risk levels, we will first give a brief summary of the pathogens present in these centres (1).

2.1. Risk group 1

This group includes bacteria, fungi, viruses and parasites with the following properties:
- There is little probability of their causing disease
- They do not have any negative effects on the environment
- They carry minimal risks for the laboratory staff.

2.2. Risk group 2

This group contains bacteria, chlamydiae, mycoplasmas, fungi, viruses and parasites with the following properties:
- They cause mild diseases
- They have moderate economic repercussions
- They have a rapid rate of biological breakdown
- Their survival is limited in the laboratory environment
- They constitute a minimal risk for the staff
- They are not transmitted horizontally
- They are easy to treat with antibiotics
- They can be prevented by using readily available means
- They are native to or endemic in the country.

2.3. Risk group 3

Pathogens falling into this group include bacteria, chlamydiae, rickettsia, fungi and viruses with the following properties:
• They can cause serious diseases
• They have large-scale negative economic consequences
• They are easily transmitted by aerosols
• They can be treated with drugs and prevented by using readily available means
• They can be prevented by using available vaccinations
• They can infect a large number of animals and humans
• They can occasionally be lethal
• They are native to or endemic in the country.

2.4. Risk group 4

This group consists of a series of viruses which have the following properties:
• They cause fairly serious diseases
• They have a high mortality rate
• They carry a risk for the staff
• They are easily transmissible by aerosols or transmitted by arthropods
• There are no any readily available vaccinations
• Medical treatment and preventive means are ineffectual and very limited
• They can be native to, endemic in or foreign to the country
• They also frequently cause diseases in humans.

3. BIOSECURITY

Biosecurity, is defined as the different methods which result in the safe handling of pathogenic micro-organisms, not only for staff in direct contact with them, but also for the people and animals around them. Biosecurity includes all the different levels of protection available when working with infectious micro-organisms.

These levels of protection can be divided into two groups:

The first level of protection concerns the staff and the environment inside the laboratory; it results from the reliable, correct accomplishment of the prescribed formalities and use of adequate microbiological techniques, as well as efficient use of the corresponding materials and equipment. The use of immunogens also largely contributes to reinforcing the level of protection for staff.

The second level of security concerns protection of the environment outside the laboratory which is achieved through a combination of the design of the installation and the working methods used.

To sum up, it can be said that the aim of biosecurity is to reduce the exposure of laboratory staff to pathogens, and to prevent potentially dangerous agents from leaving the laboratory.

4. FORMALITIES AND LABORATORY TECHNIQUES

The most important safety factor is the correct accomplishment of working practices in the laboratory itself. People in contact with infectious agents and infected equipment must be aware of the potential dangers involved; they must be given solid training in respecting the practices required for the safe handling of dangerous equipment.

Each laboratory must develop and adopt a set of biosecurity standards which define both the potential risks and the practices and procedures required to reduce or eliminate them. A person in charge of biosecurity should be appointed and a committee set up to revise the standards and ensure that they are respected.

5. BIOSECURITY EQUIPMENT

5.1. HEPA filters
Developed in the 1940s to create dust-free areas, High Efficiency Particulate Air (HEPA) filters are made from a single sheet of borosilicate fibre divided by corrugated aluminium separators. They can filter particles greater than or equal to 0.3 µm, which essentially includes all bacteria, spores and viruses, at an efficiency rate of 99.97%. HEPA filters are fairly fragile, and can be damaged if they are dropped or not looked after properly (4).

5.2. Biosecurity Cabinets

There are three types of biosecurity cabinets: Class I, Class II and Class III (4). Their main characteristics are as follows:

5.2.1. Class I Biosecurity Cabinets

These protect the staff and environment, but do not protect the product. They operate on a principle similar to that of air passing through a chemical gas extractor hood, but they have a HEPA filter in the air outlet system to protect the environment.

Because Class I cabinets do not protect the product, they are frequently used to enclose equipment such as centrifuges and fermenters.

5.2.2. Class II Biosecurity Cabinets:

Class II cabinets are based on the principle of laminar flow and protect the staff, the environment and the product. Class II cabinets send the ambient air around the operator to the front grille of the cabinet, thus protecting the staff. Also, the descending laminar air flow filtered through a HEPA filter protects the product, reducing the probability of contamination in the work station inside the cabinet. Because the exhaust air is filtered through a HEPA filter, it is uncontaminated and can be recycled into the laboratory or evacuated outside the building.

There are four types of Class II cabinets, types A, B1, B2 and B3. These are described in detail below.

Class II, Type A Cabinet

- The air in the room is taken through the front of the cabinet at a minimum speed of 75 linear feet per minute.
- Thirty percent (30%) of the air leaves through a HEPA outlet filter and is recycled into the laboratory. Seventy percent (70%) of the air passes through a HEPA filter and is returned to the work station.
- Class II A cabinets should not be used to handle volatile or toxic chemicals if the air outlet system is not connected to a duct which takes it outside the building.

Class II, Type B1 Cabinet

- The air in the room is taken through the front of the cabinet at a minimum speed of 100 linear feet per minute.
- Seventy percent (70%) of the air passes through the grille at the rear of the cabinet and leaves through a HEPA outlet filter. Thirty percent (30%) of the air passes through the front grille and through a HEPA filter and is then returned to the work station.
- Laboratory work which produces dangerous or particle chemical fumes must be carried out at the back of the cabinet.

Class II, Type B2 Cabinet

- Air from the room is taken through the upper part of the front of the cabinet at a minimum speed of 100 linear feet per minute.
- All the air absorbed is discharged through a HEPA filter.

Class II, Type B3 Cabinet

- This is a type 1 cabinet connected to an outlet duct. The air inlet speed at the front of the cabinet is 100 linear feet per minute.

5.2.3. Class III Biosecurity Cabinets
These cabinets are designed to provide maximum protection for the environment and staff when handling risk group 4 pathogens.

Their main characteristics are as follows:

- They are completely sealed and airtight.
- Access is through autoclaves with double doors or disinfecting tanks.
- Large rubber gloves are added to the front of the cabinet, for handling equipment which is isolated from each other.
- Both the incoming and outgoing air passes through HEPA filters. The outgoing air passes through two HEPA filters, or a HEPA filter and an air incinerator.
- The Cabinet is kept at a negative pressure of 0.5 inches of water.

6. USE OF HORIZONTAL OR VERTICAL FLOW CABINETS

Horizontal and vertical flow cabinets ("clean benches") are not biosecurity cabinets and must not be used as such for investigation applications and in veterinary and medical laboratories.

"Clean benches" protect only the product and must not be used to handle tissue cultures or potentially infectious equipment. They are regularly used for equipment assembly work and electronic appliances where product sterility and cleanliness are the most important aspects.

7. MINIMUM BIOSECURITY REQUIREMENTS FOR THE DIFFERENT LEVELS OF RISK

7.1. Risk level 1

7.1.1. Operating methods

- Biosecurity cabinets are not required to handle risk group 1 diseases.
- The use of laboratory coats, laboratory aprons or uniforms is recommended to prevent contamination or staining of outdoor clothing.
- Gloves must be used if the skin is scratched or irritated.
- Protective glasses must be used if there is any risk of dangerous materials or micro-organisms being splashed onto the face.

7.1.2. Laboratory facilities

- Each laboratory must have a wash basin.
- The room must be separated from public areas or corridors used for other traffic by a permanently closed door.
- The laboratory must be easy to clean, especially paint or coverings on walls, ceilings, furniture and floors.
- If the windows can be opened, they must not be near the work stations or containment equipment. They must have fly screens.
- There are no requirements for special air control systems, except those which are necessary for correct operation of the biosecurity cabinets.
- A wash basin must be available near laboratory exits which lead to public areas.
- Separate areas must be provided to change from outdoor clothing into laboratory coats.

7.2. Risk level 2

Requirements for risk level 2 laboratories, are the same as those described for risk level 1 laboratories with the addition of the conditions listed below.

7.2.1. Operating methods
• Class I and II biosecurity cabinets are required in order to handle agents which can create aerosols.
• The air from the cabinets can be recycled into the laboratory provided that it passes through a HEPA filter first.
• Centrifuges must be kept closed or covered or completely sealed off from aerosols. They must only be opened inside a biosecurity cabinet.
• Animals and insects which have been infected experimentally must remain inside the laboratory or in animal containment units.
• A written emergency plan to deal with the leakage of infectious pathogens must be established and used when necessary. The laboratory staff must be trained and skilled in emergency action.
• The vacuum equipment used to work with pathogens must be protected with HEPA filters and traps containing disinfectant fluids.
• Laboratory coats must only be worn in the laboratory. They must be washed in the laboratory by the institution. They must not be taken away by the staff to be washed at home or in laundromats.
• Gloves must be used to avoid contact with infected materials or animals. Special attention must be paid if the skin is damaged or irritated.
• Protective glasses must be used to prevent dangerous materials or micro-organisms from being splashed onto the face.
• Contaminated glassware must be decontaminated before being removed from the laboratory. If there are no autoclaves or incinerators in the laboratory, the contaminated equipment must be disinfected chemically or packed in bags with an inner lining and transported to an autoclave or incinerator in sturdy leak-proof containers which must be closed and disinfected on the outside before leaving the laboratory.
• Any cleaning and maintenance staff who enter the laboratory must be informed of the dangers to which they may be exposed.
• The cleaning staff must only clean the floors. The laboratory staff is responsible for keeping the premises safe by routine cleaning. Detailed cleaning schedules must be drawn up at regular intervals.
• The cleaning and maintenance staff must be given medical check-ups and immunised when necessary.

7.2.2. Physical requirements

• Each laboratory must have a sink.
• There must be a special place to wash the eyes.
• The laboratory must be designed so that it is easy to clean.
• The laboratory must be kept separate from public areas and general offices.
• Biological risk signs must be posted up at the entrance to the laboratory giving all necessary information.
• Laboratory furniture and work benches must be water-proof and easy to clean.
• Hooks to hang up laboratory coats must be provided near the exit.
• An autoclave must be available in or near the laboratory.
• The laboratory doors must close automatically or pneumatically.

7.3. Risk level 3

Biosecurity requirements for risk level 3 diseases are far more stringent than they are for risk levels 1 and 2. The laboratory staff must be given special training in handling and manipulating the pathogens used in the laboratory. Because laboratories which handle level 3 diseases are designed to keep the leakage of dangerous materials outside the laboratory to a minimum and provide additional protection for the staff, these laboratories must undergo a series of tests each year and their biosecurity conditions must also be checked.

A level 3 laboratory must be especially designed and built. The people in charge of biosecurity in the country concerned must maintain strict control, seek experienced assistance and keep in continual contact throughout the design, construction, inspection, testing, operation and maintenance of the laboratory, including annual testing.

7.3.1. Operating methods

As well as the operating methods required for level 1 and 2 laboratories, the following are required:
• Class II and III biosecurity cabinets. These must undergo annual maintenance and certification inspections.
• Aerosol protection equipment, such as masks, safety covers for centrifuges, rotors for sealed centrifuges and animal cages, must be provided.
• Laboratory coats must be completed closed at the front or laboratory aprons used. Uniforms or laboratory coats must only be used in the laboratory. If uniforms are re-usable, they must be decontaminated before being washed.

7.3.2. Physical requirements

• The laboratory must be located away from general working zones and access to other zones must be controlled. Changing rooms must have doors with automatic closing and a key lock.
• The laboratory windows must be of the sealed, unbreakable type.
• The laboratory must be kept in a relative vacuum with regard to the surrounding areas at all times so that a directional air flow is created in the incoming area through all the entrance and exits areas.
• The laboratory must have a dedicated airtight system for incoming and outgoing air. If there is not a dedicated system, the air must be filtered through HEPA filters before the entering the main exhaust duct for the building.
• The air supply system must have a non-return valve or a HEPA filter to prevent the return of contaminated air to the supply fan or the air supply system.
• The laboratory exhaust air cannot be recycled to the laboratory’s air supply system or that of other buildings or adjacent buildings.
• At the outlet, the air must be far removed from the air inlet or populated areas.
• The biosecurity cabinets must be installed in such a way that they do not interfere with the balance of the air in the room and the cabinet.
• The laboratory must have a wash basin operated by foot pedal, knee or a presence detector; the basin must be near the exit of contaminated areas.
• The laboratory must have a movable or fixed autoclave in the working area.
• The laboratory furniture must be kept to a minimum. The work stations must be waterproof, easy to clean and withstand chemical disinfecting.
• All the service ducts in the walls, floors and ceilings must be sealed. The air supply and exhaust system must have a valve inside the room for disinfecting with gas.
• The laboratory water supply must have non-return valves.
• All the vacuum equipment, including portable equipment must have on-line HEPA filters. The vacuum equipment must not leave the contamination area.
• Emergency power must be supplied to critical equipment such as biosecurity cabinets, gas extractors and freezers.

7.4. Risk level 4

The operating practices and physical requirements of a risk level 4 laboratory are extremely specialised. Any country which carries out research in level 4 diseases must seek experienced assistance in developing appropriate laboratory designs and operating formalities. Biosecurity level 4 is the highest security level and corresponds to a functionally isolated unit operating independently of any other area. Level 4 laboratories are especially designed to prevent micro-organisms from being emitted into the environment.

7.4.1. Operating practices

• Class III biosecurity cabinets are required.
• Class II biosecurity cabinets can be used provided the staff wear individual pressurised clothing.

7.4.2. Physical requirements

• The laboratory must be physically separated from any other laboratory, or consist of a completely insulated single-piece construction, in which all wall, ceiling and floor ducts are completely airtight so that no dangerous materials can leave the laboratory.
• The laboratory must be designed for a minimum of two people, and must include all the necessary laboratory equipment to keep cultures for long periods of time and to handle infected animals.
• The entrance to the laboratory must be through electro-mechanically closed doors.
• The changing rooms must be adjacent to the contamination area. Individual or chemical showers must be provided.
• All drains must be filled with disinfectant.
• All ventilation systems for contaminated water pipes must have HEPA filters.
• The gas supply lines must have HEPA filters and non-return valves to prevent contaminated products from flowing back up the supply line.
• The laboratory must have a double-door autoclave on which maintenance can be carried out from outside the security area.
• The laboratory must have two-way intercommunication and closed circuit camera systems.
• Residual water must pass through a sterilisation system which is chemically and biologically monitored.
• The structure must be ventilated by an independent system with a dedicated, airtight supply and exhaust system. The air must not be recycled.
• The exhaust air must pass through two series-mounted HEPA filters located in an airtight chamber to allow for decontamination and certification.
• The air supply system must have a HEPA filter.
• The system must have an emergency power supply for all alarms, ventilation systems and other critical equipment.

8. SURVEY RESULTS

The authors thank the administrations of the Veterinary Services of the countries which completed the questionnaire: Argentina, Brazil, Bolivia, Canada, Chile, Colombia, Costa Rica, Mexico, Panama and Paraguay.

The information provided by these countries on the main pathogenic micro-organisms handled by veterinary laboratories concerned with antigen and vaccine diagnostics and preparation is summarised up in Table 1.

A comparison of the types of pathogens handled in these laboratories and their biosecurity equipment and physical installations shows that only four laboratories fulfil the minimum biosecurity requirements for the handling of pathogens from risk group 3. The remaining laboratories which handle pathogens from this same risk group are deficient in one or more of the following respects:

• They do not use biosecurity cabinets: they use horizontal or vertical laminar flow cabinets.
• They do not have centrifuges containing aerosols.
• There are no showers between contaminated areas and clean areas.
• There is not a double door laboratory access system.
• There are no work stations which are impermeable to water and resistant to the action of acids and alkalis, organic solvents and moderate heat.
• The uniforms used in the laboratories are not decontaminated before being washed.
• They do not have ventilation systems which ensure directional air flow.
• They do not have single passage ventilation systems or do not use HEPA filters to disinfect the air.
• They do not have negative pressure control systems in the laboratory.

Laboratory handling pathogens of risk level 1 and 2, with the exception of those using horizontal and vertical laminar air flow cabinets, have neither double entry doors nor water-proof work areas which are resistant to acids, alkalines, organic solvents and moderate heat. Such laboratories are appropriate for the handling of pathogens of theses groups.

<p>| Table 1: Main pathogenic micro-organisms to be found in veterinary laboratories which make diagnoses and prepare antigens and vaccines |
|-----------------|-----------------|-----------------|
| Viral diseases  | Bacteria         | Fungi           |
| Equine infectious anaemia | Actinobacillus | Aspergillus     |
| Avian infectious bronchitis  | Bacillus anthracis | Mucor               |
| Coronavirus      | Brucella canis  | Rhizopus        |
| Bovine viral diarrhoea    | Brucellas (others) | Trichophyton |
| Equine encephalomyelitis (East, West, Venezuelan) | Clostridia |  |
| Caprine arthritis/encephalitis | Clostridium botulinum |  |</p>
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**REFERENCES**


