



SAARC Regional Training

19-24 August 2019



Training Manual

on

Laboratory Biosafety and Biosecurity for Handling Transboundary Animal Diseases and Zoonotic Emerging Pathogens

Organized by :

**ICAR - National Institute of Veterinary Epidemiology and
Disease Informatics, Yelahanka, Bengaluru - 560 064, India**



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SAARC Agriculture Centre, Dhaka, Bangladesh



Biosafety Laboratory



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SAARC Regional Training

19-24 August 2019



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SAARC Agriculture Centre, Dhaka, Bangladesh

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Laboratory Biosafety and Biosecurity for Handling Transboundary Animal Diseases and Zoonotic Emerging Pathogens

Course Director

Dr. Parimal Roy

Director, ICAR-NIVEDI, Bengaluru

Course Coordinators

Dr. Jagadish Hiremath

Dr. Manjunatha Reddy G B

Dr. R. Sridevi

Dr. Siju Susan Jocab

Organized by

**ICAR-National Institute of Veterinary Epidemiology and Disease
Informatics (NIVEDI), Yelahanka, Bengaluru, India**

Course Co-coordinators:

Dr. Mohd. Mudassar Chanda,
Dr. M. Nagalingam,
Dr. Yogisharadhya

Editors:

Dr. Parimal Roy,
Dr. Jagadish Hiremath,
Dr. Manjunatha Reddy G.B.,
Dr. Ashis Kumar Samanta,
Dr. S. M. Bokhtiar

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MESSAGE

I am immensely pleased to know that National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI) is organizing a SAARC regional training on 'Laboratory Biosafety and Biosecurity for Handling Transboundary Animal Diseases and Zoonotic Emerging Pathogens' under the aegis of SAARC Agriculture centre (SAC), Bangladesh from 19-24th August, 2019 at Bengaluru, India.

The increased global demand for improved disease detection and control in the era of emerging and re-emerging diseases has resulted in the expansion of diagnostic and research capacity. However, the increase in infectious disease detection capacity has not been paralleled by an increase in bio-safety and bio-security capacity, particularly in developing countries. In order to address this issue, understanding about the appropriate laboratory bio-safety and bio-security measures along with bio-risk analysis is required to reduce the health, safety and security risks. In global scenario, co-operation from each country in terms of maintaining strict biosafety and biosecurity measures is an inevitable element in preventing Transboundary diseases. This training program organized by ICAR-NIVEDI will help the researchers of National Veterinary Laboratories of South Asian Association for Regional Cooperation (SAARC) member countries to increase the understanding about the handling infectious agents by adhering to strict biosafety and biosecurity measures thereby minimizing the bio-risk.

I wish the training all success and complement the organizers for selecting appropriate theme for deliberation which will definitely provide cross learning opportunities within the SAARC region.

**Dated the 6th August, 2019
New Delhi**

(T. MOHAPATRA)



Indian Council of Agricultural Research
Krishi Bhavan, New Delhi - 110 001



Dr. J. K. Jena
Deputy Director General
(Animal Science)

MESSAGE

It gives me immense pleasure that National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI) is organizing a SAARC regional training on ‘Laboratory Biosafety and Biosecurity for Handling Transboundary Animal Diseases and Zoonotic Emerging Pathogens’ from 19-24TH August, 2019 under the aegis of SAARC Agriculture centre (SAC), Bangladesh.

Globally, addressing the emergence and spread of infectious diseases and ensuring the safe handling and secure storage of pathogens in laboratories is a matter of much debate. It demands that each country need to have the ability or resources to execute appropriate bio-safety and bio-security principles and practices to laboratory operations to effectively control trans-boundary and emerging diseases thereby ensuring the safety of each country. In this regard, capacity building programs will facilitate the countries to join hands together by imparting knowledge to each other to strictly adhere to laboratory bio-safety, bio-security and risk management measures thereby effectively and efficiently preventing the spread of infectious disease within and between countries.

I wish this endeavour a grand success.

(J.K. Jena)





DIRECTOR,
SAARC AGRICULTURE CENTRE

I am happy to know that the manual is being published for the SAARC Regional Training Programme on “Laboratory Biosafety and Biosecurity for handling Transboundary Animal Diseases and Zoonotic Emerging Pathogens” jointly by SAARC Agriculture Centre (SAC), Dhaka, Bangladesh and ICAR – National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI), Bangalore, India scheduled to be held from 19th to 24th August, 2019.

SAARC Agriculture Centre (SAC), under the tutelage of South Asian Association for Regional Cooperation (SAARC) has been working for the promotion of agricultural research & development as well as technology transfer through regional networks among agricultural research/ extension institutions and policy makers in the SAARC region and livestock is considered as one of the priority sector. ICAR- NIVEDI is one of the prestigious institute working under the umbrella of Indian Council of Agricultural Research on numerous animal diseases and informatics, including transboundary and zoonotic pathogens. In line with the FAO/OIE action plan on “Global Framework for Progressive Control of Transboundary Animal Disease (GF-TADs)”, the SAARC Agriculture Centre is glad to carry out the Regional Capacity Development programme for the Member States to order to mitigate the challenges of transboundary animals disease/ zoonotic emerging pathogens.

I hope that the skills and experiences acquired in this Regional Training Programme will help the participants of the National Agricultural Research and Extension Systems of SAARC Member States to perform better for planning and execution of strategies pertaining to the issues of transboundary disease of animal origin. Evidently, it will strengthen the fight against transboundary animal diseases in South Asia collectively and scientifically.

I wish the training manual will act as primary guiding force for the participants for handling transboundary animal diseases and zoonotic emerging pathogens during their future research or control measures.

I wish all the grand success for this regional training programme and its endeavors.

Dated: 9th August, 2019

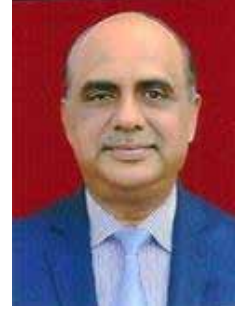
Dhaka, Bangladesh

Dr. S. M. Bokhtiar



**Indian Council of Agricultural Research
Krishi Bhavan, New Delhi - 110 001**

Telefax (O) : 011 23386668 Email: ashokkr.icar@gov.in, ashokakt@rediffmail.com



Dr Ashok Kumar
Assistant Director General (Animal Health)
&
President, Indian Association of
Veterinary Public Health Specialists

MESSAGE

I am immensely delighted to know that ICAR- National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI), Bengaluru is organizing a SAARC regional training on 'Laboratory Biosafety and Biosecurity for Handling Transboundary Animal Diseases and Zoonotic Emerging Pathogens' from 19-24th August, 2019 under the aegis of SAARC Agriculture centre (SAC), Bangladesh.

There is global recognition that emerging animal-borne diseases can be expected in the future and handling the infectious uncertainty has become an imperative. Tackling this urgent situation demands the co-operation at regional level with sound biosafety and biosecurity measures. To achieve this objective, capacity building programmes will help to strengthen the ability of countries to control transboundary diseases by maintaining appropriate biosafety and biosecurity measures along with risk assessment and management of identified risks.

I wish all the success for this training.

(Ashok Kumar)



भाकृअनुप - राष्ठीय पशुरोग जानपदिक एवं सूचना विज्ञान संस्थान
ICAR-National Institute of Veterinary Epidemiology and Disease Informatics

रामगोडनहल्लि, येलहंका, बेंगलुरु - 560 064, भारत
Ramagondanahalli, Post Box No. 6450, Yelahanka, Bengaluru - 560 064, INDIA

Dr. Parimal Roy, M.V.Sc., Ph.D.
Director



Message

ICAR-NIVEDI, is a pioneer research institute under Indian Council of Agricultural Research (ICAR), has been entrusted to conduct Research & Development in the field of Veterinary Epidemiology and animal disease surveillance for the entire country.

Climate change and global warming paved the way to emergence of infectious disease that have potential for transboundary transmission. Countries that failed to implement strict biosafety and biosecurity practices have incurred huge economic loss. In this context laboratory capacity and laboratory biosecurity are foremost consideration for identifying pathogens and their biocontainment. ICAR-NIVEDI has BSL2+ laboratory with strict biosafety and biosecurity practices as per the guidelines of DBT.

ICAR-NIVEDI has been given the responsibility to host the SAARC sponsored training on Laboratory Biosafety and Biosecurity for Handling Transboundary Animal Diseases and Zoonotic Emerging Pathogens' from 19-24th August, 2019 under the aegis of SAARC Agriculture centre (SAC), Bangladesh. This training will be mutually beneficial to the participants as well as to the host faculty to understand strength and weaknesses of member countries in the area of biosafety and biosecurity. This training will create an avenue for ICAR-NIVEDI to deliver our experience in the field of laboratory biosafety, biosecurity, risk assessment and management of bio-risks which will pave a better way to control the transboundary animal diseases and emerging zoonotic infections within and between the SAARC countries.

I wish the participants a pleasant stay and I wish the training a great success

A handwritten signature in green ink, appearing to read 'Parimal Roy', written in a cursive style.

(Parimal Roy)



Training Schedule

SAARC Regional Training on **“Laboratory Biosafety and Biosecurity for Handling Transboundary Animal Diseases and Zoonotic Emerging Pathogens”** 19-24th August 2019 at ICAR-NIVEDI, Bengaluru, India

Day	Time	Topic	Resource person/s
19/08/2019 Monday	9.30 -10.00am	Registration	
	10.00-11.00am	Inauguration	
	11.00- 11.30am	High tea	
	11.30-12.00pm	About ICAR-NIVEDI	Dr. Parimal Roy, Director, ICAR-NIVEDI
	12.00-12.30pm	Training Introduction and Pre-Test:	Dr. Parimal Roy, Dr. Jagadish Hiremath
	12.30-1.00pm	Introduction to Biosafety and Biosecurity	Dr. Jagadish Hiremath
	1.00-2.00pm	Lunch	
	2.00-2.30pm	Laboratory Premises	Dr. G. B. Manjunatha Reddy
	2.30-3.00pm	Good Laboratory Practices (GLP)	Dr. Yogisharadhya Dr. Siju Susan Jacob
	3.00-3.15	Break	
	3.15-4.30pm	Safety Levels and Agent Classification (Theory and Practical)	Dr. Jagadish Hiremath
20/08/2019 Tuesday	9.30-10.30am	National Biosafety Regulations in SAARC Member Countries	Dr. A. K. Rawat, Scientist G, DBT, India Dr. Jagadish Hiremath
	10.30-11.00am	Personal Protective Equipment (PPE)	Dr. R. Sridevi
	11.00-11.15am	Break	
	11.15-12.00pm	PPE (Practical)	Dr. Jagadish Hiremath Dr. R. Sridevi
	12.00-1.00pm	Signages (Theory and Practical)	Dr. Jagadish Hiremath Dr. S. S. Patil
	1.00-2.00pm	Lunch	
	2.00-3.15pm	Disinfection & Antiseptics: Laboratory Cleaning & Hand Washing: (Theory and Practical)	Dr. R. Sridevi Dr. Jagadish Hiremath
	03.15-03.30pm	Break	
	3.30-4.30pm	Biosafety Cabinets: Types, Practices and Safe Behaviours (Theory and Practical)	Dr. Manjunatha Reddy G. B Dr. Jagadish Hiremath

21/08/2019 Wednesday	9.30-10.30am	Emergencies in biosafety lab and ways to handle them (Theory and Practical)	Dr. Jagadish Hiremath
	10.30-11.15am	Laboratory waste management: Autoclave and ETP (Theory and Practical)	Dr. M. Nagalingam Dr. R Sirdevi Dr. Jagadish Hiremath
	11.15-11.30am	Break	
	11.30-12.30pm	Biosafety documentation, SOP and Manual (Theory and Practical)	Dr. D. Hemadri Dr. Jagadish Hiremath
	12.30-1.00pm	Biosafety practices: Collection, Package and Transportation of Infectious Materials- National (Theory).	Dr. P. Krishnamoorthy
	1.00-2.00pm	Lunch	
	2.00-2.30pm	Biosafety practices: Collection & Transportation of Infectious Materials- International (Theory)	Dr. Manjunatha Reddy G B
	3.00-3.15pm	Break	
	3.15-3.45pm	Biosafety practices: Collection & Transportation of Infectious Materials (Practical)	Dr. Jagadish Hiremath Dr. Avadesh Prajapati
	3.45-4.45pm	Biosecurity: Physical, Personal, Pathogen and data security (Theory and Practical)	Dr. Jagadish Hiremath
22/08/2019 Thursday	9.30-10.30am	Biosecurity: Case studies and discussion points	Dr. Jagadish Hiremath
	10.30-11.15am	Laboratory biosecurity program	Dr. Jagadish Hiremath
	11.15-11.30am	Break	
	11.30-12.00pm	Laboratory biosecurity program cont...	Dr. Jagadish Hiremath
	12.00-12.30pm	Agent Specific Biosafety and Biosecurity Practices: <i>Rabies</i>	Dr. G. B. Manjunatha Reddy
	12.30-01.00pm	Agent Specific Biosafety and Biosecurity Practices: <i>Brucella spp.</i>	Dr. R. Shome
	1.00-2.00pm	Lunch	
	2.00-2.30pm	Biosecurity and Biosafety practices in surveillance of emerging tick borne diseases	Dr. Mohd Mudassar Chanda Dr. Jagadish Hiremath
	2.30-3.00pm	Agent Specific Biosafety and Biosecurity Practices: <i>Parasitic Diseases</i>	Dr. P. P. Sengupta Dr. Siju Susan Jacob
	3.00-3.15pm	Break	
	3.15-4.00pm	Agent Specific Biosafety and Biosecurity Practices: <i>Bacillus Anthracis</i>	Dr. Satish B Shivachandra Dr. Jagadish Hiremath
	4.00-4.30pm	Agent Specific Biosafety and Biosecurity Practices: Transboundary Animal Pathogen- <i>African swine fever virus</i>	Dr. S. S. Patil Dr. Jagadish Hiremath
	4.30-5.30pm	Agent Specific Biosafety and Biosecurity Practices: <i>Leptospira spp.</i>	Dr. V. Balamurugan

23/08/2019 Friday	9.30-5.00pm	Field Visit	Dr. Yogisharadhya Dr. Awadesh Prajapathi
24/08/2019 Saturday	9.30-10.30am	Agent Specific Biosafety and Biosecurity Practices: <i>Glanders</i>	Dr. Praveen Malik Animal Husbandry Commissioner (Offctg) Director, CCS National Institute of Animal Health,
	10.30-11.30am	Risk management in laboratory (Theory)	Dr. H. V. Murgkar Principle Scientist, ICAR- NIHSAD, Bhopal, MP, India Dr. Jagadish Hiremath
	11.30-11.45am	Break	
	11.45-01.00pm	Risk management in laboratory (Practical)	Dr. Jagadish Hiremath
	1.00-2.00pm	Lunch	
	2.00-3.00pm	Preparation and presentation of plan of action by Participants	Dr. Jagadish Hiremath Dr. R. Sridevi Dr. G. B. Manjunatha Reddy Dr. Yogisharadhya
	3.00-3.30pm	Post-Test and Feedback	Dr. Jagadish Hiremath Dr. R. Sridevi Dr. G. B. Manjunatha Reddy Dr. Yogisharadhya
	3.30-4.30pm	Valedictory	



About the Training Manual

We have developed this manual to facilitate the trainees to understand and practice the concepts of Laboratory Biosafety and Biosecurity to conduct a safe research that involves handling of transboundary animal diseases and zoonotic emerging pathogens. The manual brings in innovative ways of demonstrating practically the concepts like safety levels, agent classification, PPE, signages in biosafety labs, emergencies, risk assessment, biosecurity etc.,

Each practical is divided in to sections like introduction, learning objectives and activity. The activities are planned to engage the trainees actively into learning process through demonstrations, group work, case studies and scenarios, hands on activity, short presentations and question and answer to create an objective understanding of the biosafety and biosecurity concepts in laboratories.

The porous borders shared by the SAARC member countries pose a challenge to contain the disease nationally or protect the disease-free nation against possible incursion of infectious agents across borders. In this context having good laboratory biosafety and biosecurity practices is important and in this regard the training manual developed will facilitate the trainees to improve their own biosafety and biosecurity practices and further develop the national plans for laboratory biosafety and biosecurity plan.



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1. Introduction to Biosafety and Biosecurity

Jagadish Hiremath

ICAR-National Institute of Veterinary Epidemiology and Disease Informatics

Concepts of biosafety and biosecurity deal with related but distinctly different issues. Laboratory biosafety refers to all the measures to protect self, people around and environment against the biological risk posed by handling of various biohazards in laboratory conditions whereas biosecurity has divergent meaning depend upon the settings.

The laboratory biosecurity describes the protection, control and accountability for valuable biological material (VBM) within the laboratory conditions with aim of preventing their unauthorized access, loss, theft, misuse, diversion or intentional release. The laboratory biosecurity program employs administrative, regulatory and physical security procedures to achieve its goal.

Laboratory biosafety involves the containment principles, technologies and practices that are implemented to prevent unintentional exposure to pathogens and toxins, or their accidental release. Depend upon the nature of agent handled and risk group categorization, a containment requirement for safe handling of the agent is developed. There are different levels of controls put in place to regulate the movement of materials and personnel in to and outside of the biosafety laboratories.

The most important element of containment is strict adherence to standard microbiological practices and techniques. Persons working with infectious agents or potentially infected materials must be aware of potential hazards, and unless proficient in the practices and techniques required for handling such material safely, one should not indulge in to such works.

Laboratory biosecurity plan is must to prevent intentional exposure or release of biohazard agents or toxins. The biosecurity is implemented at different layers covering physical, personal, pathogen and data security.

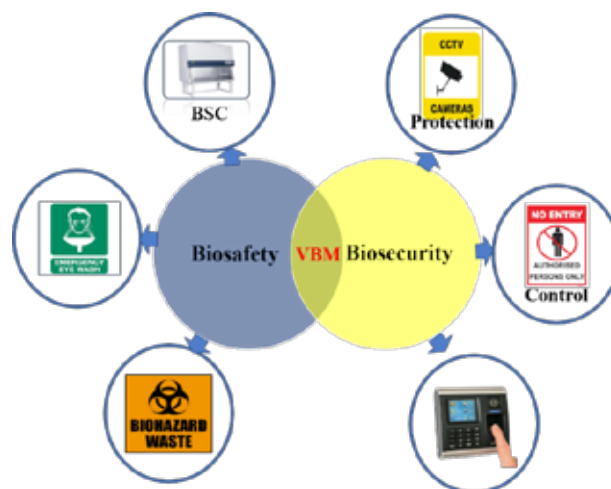


Fig.1 Laboratory Biosafety and Biosecurity

2. Laboratory Premises

G. B. Manjunatha Reddy

ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI)

A 'good' laboratory is essential for providing a 'good' diagnostic service to the animal stakeholders. To build a good or standard laboratory, one of the basic requirements is proper design with technical guidance. Dearth of knowledge and awareness regarding laboratory designing will create many problems in building new facilities or upgrading the existing buildings to laboratories. This might be due attributed non availability of a national guidelines in the respective countries. The safe operation of the laboratories and the safety of the laboratory personnel cannot be achieved with design compromised laboratories. Due to increasing trends in emergence of new pathogens, the biosafety laboratories have become a integral part of national health management and also very sensitive issue. Therefore there is need for formulating the national technical guidelines on the design and management of the laboratories and improve the laboratory infrastructure to meet with international standards of laboratory safety in this region of world.

- Laboratory - the room in which biological agents are handled.
- Laboratory suite - one or more laboratories, not necessarily of the same discipline or containment level, and ancillary rooms within a section or department.
- Laboratory unit - separate building or self-contained suite within a building containing one or more laboratories and with ancillary rooms.
- Biocontainment Laboratory - A laboratory that has been designed to lessen or completely prevent the escape of microorganisms to protect employees, public, research and environmental health.

Types of Biocontainment Laboratories

Biological agents need to be managed in the laboratory environment so as to prevent / control the exposure of laboratory workers, other people and the outside environment to these agents. There are different levels of biosafety laboratories depending on Risk groups, biosafety levels, practices and equipment (Table)

BSL	Laboratory type	Laboratory practices	Safety equipment
1	Basic teaching, research	Good microbiological techniques	None Open bench work
2	Primary health services; diagnostic services, research	Good microbiological techniques, protective clothing, biohazard sign	Open bench PLUS biological safety cabinet for potential aerosols
3	Special diagnostic services, research	As BSL 2 PLUS special clothing, controlled access, directional airflow	Biological safety cabinet and/or other primary devices for all activities
4	Dangerous pathogen units	As BSL 3 PLUS airlock entry, shower exit, special waste	Class III biological safety cabinet, positive pressure suits, double ended autoclave (through the wall), filtered air

General Principles of Designing

1. Designing process: Collaborative work of the client and the designer.
2. Designing committee: Architect, Administrative Head of the Institution, Construction Engineer, Scientists of institute/Consultant Microbiologist and Project Coordinator.
3. General design considerations: There are a number of factors which need to be considered as appropriate, some of them are as follows.
 - Number of users
 - Space and function proportion
 - Using centralized instrumentation facilities
 - Comfortable working environment
 - User friendliness
 - Health and safety issues

Above issues should be matched with expected site of the laboratory, size/dimensions, sections and interior, commissioning and maintenance.

4. Specific Design considerations: These are very crucial for achieving the goals set for the laboratory, which includes
 - Laboratory layout
 - Laboratory type (BSL-2)
 - Types of rooms/areas: The rooms/areas should be designated based on sample reception, personnel (male and female) entry, instrument entry, building management, general and special laboratory space, common facilities, washing, autoclave and media preparation, storage and relax rooms, toilets etc., its generally advised to separate the wet lab with other spaces like offices and administrative support buildings, conference / teaching facilities, cafeterias, medical facility, hostel, staff welfare etc.,.
 - Air handling /Ventilation and cooling systems.
5. Other Important aspects to consider in designing a BSL2 laboratory includes
 - Work flow and emergency facilities
 - Types of walls and flooring systems
 - Water and power supply
 - Housekeeping / cleaning
 - Laboratory waste management
 - Physical and personnel safety and security

Factors like compromise in basic bio-safety principles (eg., inadvertent handling of the infectious material), inappropriate decontamination procedures/protocols and improper laboratory waste disposal, lack of knowledge about prudent practices in the laboratory, lack of dedicated manpower, unaccountability, improper and less efficient facilities, interrupted power supply and lack of timely supply and replenishment of needy resources, absence of strict enforcement of laboratory discipline pose great risk of chances of leakage of infectious pathogens to the exterior resulting exposure of the researcher/lab personnel and environmental contamination resulting in jeopardizing the community at large and national security critically. Hence, safe laboratory design, security, strict adherence to standard operating procedures, personal conduct, awareness of the agent being handled and reporting of laboratory accidents are paramount in ensuring safe, responsible and fool proof system of research and education.

3. Good Laboratory Practices (GLP)

Siju Susan Jacob

ICAR-National Institute of Veterinary Epidemiology and Disease Informatics

Good laboratory Practice (GLP) embodies a set of principles that provides a framework within which laboratory studies are planned, performed, monitored, recorded, reported and archived. The objective of this Code of Practice is to provide a standard for good laboratory practice when working with chemicals rather than attempt to be specific regarding either substances or procedures. It is of paramount importance that laboratory workers undertake their work and conduct themselves in a manner that ensures that exposure to chemical, biological and physical are hazardous to human health.

Three levels of protection in the laboratory

A fundamental objective of any biosafety program is the containment of potentially harmful biological agents. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other persons, and the outside environment to potentially hazardous agents.



- Installation design
- Safety equipment
- SOPs and safety measures

1. Installation design: The design and construction of the facility contributes to the laboratory workers' protection, provides a barrier to protect persons outside the laboratory, and protects persons or animals in the community from infectious agents that may be accidentally released from the laboratory.

2. Safety Equipment: Safety equipment includes Bio Safety Cabinets (BSC), enclosed containers, and other engineering controls designed to remove or minimize exposures to hazardous biological materials. The BSC is the principal device used to provide containment of infectious splashes or aerosols generated by many microbiological procedures. Safety equipment also may include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles.

3. SOPs and safety measures: Each laboratory should develop or adopt a biosafety or operations manual that identifies the hazards that will or may be encountered, and that specifies practices and procedures designed to minimize or eliminate exposures to these hazards.

Safety measures:

Prudent practices and sound techniques are of extreme importance in laboratory safety. Both are based on technical knowledge, experience, common sense, and an attitude of courtesy and consideration for others.

- Personal clothing and baggage should be left in lockers or hanging up outside the laboratory.
- Do not eat, drink, chew, store food or drink, smoke, or apply cosmetics in the laboratory.
- Never pipette anything by mouth - use a bulb or pipette gun.
- Always wear laboratory coats and properly fastened will provide protection against spillages and falling objects when working at the bench.
- Remove any wrist watches or adornments that may be exposed to hazardous agents before starting work.
- Always cover any cuts or grazes with a suitable waterproof dressing before working in a laboratory.
- Under no circumstances should gloves that have been used for bench work (radioactivity, bacteria, blood, ethidium bromide etc.) be allowed to come into contact with door handles, switches, taps, telephones, or other such communal items that will be used by laboratory staff with bare hands.
- Transport samples between laboratories in an appropriate container and use bare hands for opening doors.
- Decontaminate/clean work surfaces before and after use and especially after spills.
- Lab coats must be decontaminated (bleached) and washed regularly
- Handle infectious materials as determined by a risk assessment. Airborne transmissible infectious agents should be handled in a certified Biosafety Cabinet (BSC) appropriate to the biosafety level (BSL) and risks for that specific agent.
- Be aware of laboratory signs and symbols

Principles of good practice

The good lab practice includes technical proficiency of lab personnel, hazard awareness training of lab personnel, Personal Protective Equipment, biohazard warning signs, minimization of aerosols during work process, hand washing, pest control measures, Biohazard waste management. Four basic principles are considered the corner stones of good practice in all laboratory work

1. Forward planning: The potential hazards associated with laboratory work related to each projects, including, crucially, the infectious agents and the hazards posed by any chemicals being used, or produced, should be determined well before the commencement of the project.

2. Risk assessment, minimise risk and control exposure to infectious agents: The aim of the risk assessment process is to minimise the risk to persons health or safety from physical hazards e.g. glass, fire, explosion, etc., and from health hazards e.g. infectious agents, chemical vapours, fume, dusts, toxic powders, corrosives, etc. by implementing risk control measures e.g. intrinsically safe

equipment, use of fume cupboards, use of suitable protective clothing and equipment, etc. The risk assessment process must result in the development of a Safe Operating Procedures (SOP), a set of rules, that is conveyed to the persons involved in the project(s), that they must follow in order to ensure, so far as is reasonably practicable, the health and safety of not only themselves but that of others who may be affected by what they do. All bio hazardous or infectious materials should be sterilized before being washed and stored or discarded. The infectious solid waste generated in infectious laboratory should be segregated and placed in red colored bag for proper disposal. The liquid waste should be decontaminated with proper decontamination protocol as per SOPs. Autoclaving is the preferred method of sterilization of the infectious materials, every personnel working with bio hazardous material should be responsible for its sterilization before disposal. To minimize hazard to emergency response personnel, all bio hazardous materials should be placed in an appropriately marked refrigerator or incubator and sterilized and it should not be placed in autoclaves overnight in anticipation of autoclaving the next day or decontamination will be followed by other personnel in the laboratory.

3. Do not underestimate the risk: Once the risk is assessed, the safety precautions need to be in place even though the level of risk is low.

4. Be prepared for emergency situations: Everyone who works in a laboratory must know the action to take should something untoward occur, such as spillage of infectious materials, or unintentional release of gas or vapour. All laboratory workers must be familiar with the local emergency procedures; they should know the location of: the nearest fire alarm point, fire extinguishers, the nearest telephone and the emergency telephone numbers that are to be used.

4. Safety Levels and Agent Classification

Jagadish Hiremath

ICAR-National Institute of Veterinary Epidemiology and Disease Informatics

Risk group categorization of biohazard agent is the basis for adopting appropriate biosafety and biosecurity practices under laboratory and field conditions. The primary factors to consider selection of safety precautions fall into two broad categories: agent hazards and laboratory procedure hazards. It is necessary first to assess the risk from a pathogen, so that it can be assigned to a Risk Group (RG). Based on the proposed work involving defined procedure will further be basis for determining the appropriate containment level. To assess the risk to humans and animals from a particular pathogen it is necessary to know whether infection with that organism can cause clinical disease and/or mortality in humans and animals, and whether it could then spread to cause disease in the general human and/or animal population. Known occurrence of human and animal infection with the organism or related organisms with similar characteristics, any history of laboratory-acquired infection, infective dose and disease severity; production of toxins or allergens are other factors to be considered for agent classification. The origin of the sample, for example samples from wildlife species may contain human or animal pathogens not normally encountered.

The volume of culture to be handled and the concentration of the organism likely to be present (Procedures such as antigen or vaccine production that require large quantities of organisms usually carry a higher risk than attempted isolation procedures).

The history of the isolate being handled. Pathogens on primary isolation or of low passage level are often more dangerous than pathogens of high passage level. In some cases, pathogenicity may be enhanced by passage or subculture using different media. The possibility of aerosol formation should be especially taken into consideration when handling fluid samples or, for example, during grinding, homogenisation and centrifugation. Additional precautions for handling and storage are required for animal disease agents from foreign countries.

Physiological state of the employees, For example, in the case of pregnancy, immunodeficiency or allergy, special precautions may be required. Sometimes certain individuals have to be excluded from particular types of work that would be especially hazardous to them.

A higher level of risk may arise when agents such as Brucella or Mycobacterium are inoculated into animals. To evaluate the impact of animal inoculation, a risk assessment should be conducted and the following factors should be considered:

- i) Host species versus inoculated species;
- ii) Strain/treatment and concentration of the inoculum;
- iii) Route of inoculation;
- iv) Animal housing;
- v) Types of sampling during the experiment.

Some pathogens need to be transmitted by specific vectors or require intermediate hosts to complete their life cycles before they can infect animals and cause disease. In countries where such vectors or intermediate hosts do not occur, or where climatic or environmental factors mitigate against their survival, the pathogen poses a lower risk to animal health than in countries where such vectors or intermediate hosts occur naturally or could survive.

(OIE *Terrestrial Manual* 2008)

Hazardous Characteristics of an Agent

Microorganisms are grouped into four Risk Groups representing increasing risks to human health.

Group 1 – Organisms that are unlikely to cause human or animal disease.

Group 2 – Organisms that may cause human or animal disease but are unlikely to be spread in the community or animal population and for which effective prophylaxis and treatment are available.

Categorization of microorganism under Group 2 animal pathogens with following features:

- i) They do not depend on vectors or intermediate hosts for transmission.
- ii) There is very limited or no transmission between different animal species.
- iii) Geographical spread if released from the laboratory is limited.
- iv) Direct animal to animal transmission is relatively limited.
- v) Mode of transmission is primarily through ingestion, inoculation or mucus membrane route.
- vi) The need to confine diseased or infected nondiseased animals is minimal.
- vii) The disease is of limited economic and/or clinical significance.
- viii) Short-term survival in the environment and effective treatment or prevention is available.

Example:

Viruses: Influenza viruses types A, B, C other than notifiable avian influenza (NAI); Newcastle disease virus; Orf (parapox virus)

Bacteria: *Alcaligenes* spp, *Arizona* spp, *Campylobacter* spp, *Chlamydophila psittaci* (nonavian), *Clostridium tetani*, *Clostridium botulinum*, *Corynebacterium* spp, *Erysipelothrix rhusiopathiae*, *Escherichia coli*, *Haemophilus* spp, *Leptospira* spp, *Listeria monocytogenes*, *Moraxella* spp, *Mycobacterium avium*, *Pasteurella* spp, *Proteus* spp, *Pseudomonas* spp, *Salmonella* spp, *Staphylococcus* spp, *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*.

Fungi: *Aspergillus fumigatus*, *Microsporium* spp, *Trichophyton* spp.

Group 3 – Organisms that can cause severe human or animal disease and may spread in the community and/or animal population but for which there is usually effective prophylaxis and treatment.

Categorization of microorganism under Group 3 animal pathogens with following features:

- i) They may depend on vectors or intermediate hosts for transmission.
- ii) Transmission between different animal species may readily occur.
- iii) Geographical spread if released from the laboratory is moderate.
- iv) Direct animal to animal transmission occurs relatively easily.
- v) The statutory confinement of diseased, infected and in-contact animals is necessary.
- vi) The disease is of severe economic and/or clinical significance.
- vii) Prophylactic and/or therapeutic treatments are not readily available or of limited benefit.

- viii) Mode of transmission may be through the airborne route or direct contact.
- ix) Are either exotic or enzootic but are subjected to official control and that have a moderate risk of spread from the laboratory.

Example:

Viruses: Rabies virus, Equine encephalomyelitis virus (Eastern, Western and Venezuelan), Japanese B encephalitis virus, Louping ill virus.

Bacteria: *Bacillus anthracis*, *Burkholderia mallei* (*Pseudomonas mallei*), *Brucella* spp.; *Chlamydia psittaci* (avian strains only), *Coxiella burnetti*, *Mycobacterium bovis*.

Group 4 – Organisms that cause severe human or animal disease may represent a high risk of spread in the community or animal population and for which there is usually no effective prophylaxis or treatment.

- i) They may depend on vectors or intermediate hosts for transmission.
- ii) Transmission between different animal species may occur very readily.
- iii) Geographical spread if released from the laboratory is widespread.
- iv) Direct animal to animal transmission occurs very easily.
- v) Can be transmitted through casual contact or indirectly.
- vi) The statutory confinement of diseased, infected and in-contact animals is necessary.
- vii) The statutory control of animal movements over a wide area is necessary.
- viii) The disease is of extremely severe economic and/or clinical significance.
- ix) No satisfactory prophylactic and/or therapeutic treatments are available.
- x) Have a high risk of spread from the laboratory into the environment and the national animal population.

(OIE *Terrestrial Manual* 2008)

Classification of Infectious Microorganisms by Risk Group

Risk Group Classification	NIH Guidelines for Research involving Recombinant DNA Molecules 2002	World Health Organization Laboratory Biosafety Manual 3 rd Edition 2004
Risk Group 1	Agents not associated with disease in healthy adult humans.	(No or low individual and community risk) A microorganism unlikely to cause human or animal disease.

Risk Group 2	Agents associated with human disease that is rarely serious and for which preventive or therapeutic interventions are often available.	(Moderate individual risk; low community risk) A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.
Risk Group 3	Agents associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk).	(High individual risk; low community risk) A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.
Risk Group 4	Agents likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk).	(High individual and community risk) A pathogen that usually causes serious human or animal disease and can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.

(Biosafety in Microbiological and Biomedical Laboratories 5th Edition)

LABORATORY BIOSAFETY LEVELS

A fundamental objective of any biosafety program is the containment of potentially harmful biological agents. The term “containment” is used in describing safe methods, facilities and equipment for managing infectious materials in the laboratory environment where they are being handled or maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other persons, and the outside environment to potentially hazardous agents.

A. Laboratory Biosafety Level Criteria

Biosafety Level 1: is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment. BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science.

Standard Microbiological Practices/ Good Microbiological Techniques (GMT)

- The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
- Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
- Mouth pipetting is prohibited; mechanical pipetting devices must be used.
- Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented.
- Perform all procedures to minimize the creation of splashes and/or aerosols.
- Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
- Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method.
- A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present.
- Special containment devices or equipment, such as BSCs, are not generally required.
- Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.
- Wear protective eyewear when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses in laboratories should also wear eye protection.
- Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Wash hands prior to leaving the laboratory.

Biosafety Level 2

Biosafety Level 2 builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that: 1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures; 2) access to the laboratory is restricted when work is being conducted; and 3) all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.

Laboratory personnel must practice GMT along with Special Practices as,

- All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
- Laboratory personnel must be provided medical surveillance, as appropriate, and offered available immunizations for agents handled or potentially present in the laboratory. Each institution should consider the need for collection and storage of serum samples from at-risk personnel.

- A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
- The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.
- Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
- Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
- Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
- Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
- Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
- Animal and plants not associated with the work being performed must not be permitted in the laboratory.
- BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.
- All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices.

Safety Equipment (Primary Barriers and Personal Protective Equipment)

- Properly maintained BSCs, other appropriate personal protective equipment, or other physical containment devices must be used whenever:
- Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.
- High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.
- Protective laboratory coats, gowns, mask, or uniforms designated for laboratory use must be worn while working with hazardous materials. Remove protective clothing before leaving for non-laboratory areas, e.g., cafeteria, library, and administrative offices). Dispose of protective clothing appropriately, or deposit it for laundering by the institution. It is recommended that laboratory clothing not be taken home.

- Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories should also wear eye protection.
- Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-2 laboratory workers should: **a.** Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary. **b.** Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
- Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.

Laboratory Facilities (Secondary Barriers)

- Laboratory doors should be self-closing and have locks in accordance with the institutional policies.
- Laboratories must have a sink for hand washing. The sink may be manually, hands-free, or automatically operated. It should be located near the exit door.
- The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.
- Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
- Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
- Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
- Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they must be fitted with screens.
- BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily travelled laboratory areas, and other possible airflow disruptions.
- Vacuum lines should be protected with liquid disinfectant traps.
- An eyewash station must be readily available.
- There are no specific requirements for ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.

- HEPA filtered exhaust air from a Class II BSC can be safely recirculation back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly exhausted to the outside through a hard connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.
- A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

Biosafety Level 3

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents, and must be supervised by scientists competent in handling infectious agents and associated procedures.

All procedures involving the manipulation of infectious materials must be conducted within BSCs or other physical containment devices. A BSL-3 laboratory has special engineering and design features.

The standard and special safety practices, equipment, and facility requirements apply to BSL-3.

Special Practices for BSL 3

- All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
- Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.
- Each institution should consider the need for collection and storage of serum samples from at-risk personnel.
- A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
- The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-3 agents.
- Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
- Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
- Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
- Animals and plants not associated with the work being performed must not be permitted in the laboratory.

- All procedures involving the manipulation of infectious materials must be conducted within a BSC, or other physical containment devices. No work with open vessels is conducted on the bench. When a procedure cannot be performed within a BSC, a combination of personal protective equipment and other containment devices, such as a centrifuge safety cup or sealed rotor must be used.

Laboratory Facilities

- Laboratory doors must be self-closing and have locks in accordance with the institutional policies. The laboratory must be separated from areas that are open to unrestricted traffic flow within the building. Laboratory access is restricted. Access to the laboratory is through two self-closing doors. A clothing change room (anteroom) may be included in the passageway between the two self-closing doors.
- Laboratories must have a sink for hand washing. The sink must be hands-free or automatically operated. It should be located near the exit door. If the laboratory is segregated into different laboratories, a sink must also be available for hand washing in each zone. Additional sinks may be required as determined by the risk assessment.
- The laboratory must be designed so that it can be easily cleaned and decontaminated. Carpets and rugs are not permitted. Seams, floors, walls, and ceiling surfaces should be sealed. Spaces around doors and ventilation openings should be capable of being sealed to facilitate space decontamination.
- Floors must be slip resistant, impervious to liquids, and resistant to chemicals. Consideration should be given to the installation of seamless, sealed, resilient or poured floors, with integral cove bases.
- Walls should be constructed to produce a sealed smooth finish that can be easily cleaned and decontaminated.
- Ceilings should be constructed, sealed, and finished in the same general manner as walls.
- Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning.
- All windows in the laboratory must be sealed.
- BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily travelled laboratory areas, and other possible airflow disruptions.
- Vacuum lines must be protected with HEPA filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
- An eyewash station must be readily available in the laboratory.
- A ducted air ventilation system is required. This system must provide sustained directional airflow by drawing air into the laboratory from “clean” areas toward “potentially contaminated” areas. The laboratory shall be designed such that under failure conditions the airflow will not be reversed.
- HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer’s recommendations. BSCs can also be connected to the laboratory exhaust

system by either a thimble (canopy) connection or directly exhausted to the outside through a hard connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be certified at least annually to assure correct performance. Class III BSCs must be directly (hard) connected up through the second exhaust HEPA filter of the cabinet. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.

- A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, or other validated decontamination method).
- Equipment that may produce infectious aerosols must be contained in primary barrier devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory. These HEPA filters should be tested and/or replaced at least annually.
- Facility design consideration should be given to means of decontaminating large pieces of equipment before removal from the laboratory.
- Enhanced environmental and personal protection may be required by the agent summary statement, risk assessment, or applicable local, state, or federal regulations. These laboratory enhancements may include, for example, one or more of the following: an anteroom for clean storage of equipment and supplies with dress-in, shower-out capabilities; gas tight dampers to facilitate laboratory isolation; final HEPA filtration of the laboratory exhaust air; laboratory effluent decontamination; and advanced access control devices, such as biometrics.
- The BSL-3 facility design, operational parameters, and procedures must be verified and documented prior to operation. Facilities must be re-verified and documented at least annually.

Biosafety Level 4

Biosafety Level 4 is required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that is frequently fatal, for which there are no vaccines or treatments, or a related agent with unknown risk of transmission. Agents with a close or identical antigenic relationship to agents requiring BSL-4 containment must be handled at this level until sufficient data are obtained either to confirm continued work at this level, or re-designate the level. Laboratory staff must have specific and thorough training in handling extremely hazardous infectious agents. Laboratory staff must understand the primary and secondary containment functions of standard and special practices, containment equipment, and laboratory design characteristics. All laboratory staff and supervisors must be competent in handling agents and procedures requiring BSL-4 containment. The laboratory supervisor in accordance with institutional policies controls access to the laboratory.

There are two models for BSL-4 laboratories:

Cabinet Laboratory—Manipulation of agents must be performed in a Class III BSC; and

Suit Laboratory—Personnel must wear a positive pressure supplied air protective suit.

BSL-4 cabinet and suit laboratories have special engineering and design features to prevent microorganisms from being disseminated into the environment.

The standard and special safety practices, equipment, and facilities as BSL-3 apply to BSL-4 along with special practices as,

- All persons entering the laboratory must be advised of the potential hazards and meet specific entry requirements in accordance with institutional policies.
- Only persons whose presence in the facility or individual laboratory rooms is required for scientific or support purposes are authorized to enter.
- Entry into the facility must be limited by means of secure, locked doors. A logbook, or other means of documenting the date and time of all persons entering and leaving the laboratory must be maintained.
- While the laboratory is operational, personnel must enter and exit the laboratory through the clothing change and shower rooms except during emergencies. All personal clothing must be removed in the outer clothing change room. All persons entering the laboratory must use laboratory clothing, including undergarments, pants, shirts, jumpsuits, shoes, and gloves (as appropriate). All persons leaving the laboratory must take a personal body shower. Used laboratory clothing must not be removed from the inner change room through the personal shower. These items must be treated as contaminated materials and decontaminated before laundering.
- Removal of biological materials that are to remain in a viable or intact state from the laboratory must be transferred to a non-breakable, sealed primary container and then enclosed in a non-breakable, sealed secondary container. These materials must be transferred through a disinfectant dunk tank, fumigation chamber, or decontamination shower. Once removed, packaged viable material must not be opened outside BSL-4 containment unless inactivated by a validated method.
- Laboratory equipment must be routinely decontaminated, as well as after spills, splashes, or other potential contamination.
- Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All incidents must be reported to the laboratory supervisor, institutional management and appropriate laboratory personnel as defined in the laboratory biosafety manual. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
- Animals and plants not associated with the work being performed must not be permitted in the laboratory.
- Supplies and materials that are not brought into the BSL-4 laboratory through the change room, must be brought in through a previously decontaminated double-door autoclave, fumigation chamber, or airlock. After securing the outer doors, personnel within the laboratory retrieve the materials by opening the interior doors of the autoclave, fumigation chamber, or airlock. These doors must be secured after materials are brought into the facility. The doors of the autoclave or fumigation chamber are interlocked in a manner that prevents opening of the outer door unless the autoclave or fumigation chamber has been operated through a decontamination cycle.

- Only necessary equipment and supplies should be stored inside the BSL-4 laboratory. All equipment and supplies taken inside the laboratory must be decontaminated before removal from the laboratory.
- Daily inspections of essential containment and life support systems must be completed and documented before laboratory work is initiated to ensure that the laboratory is operating according to established parameters.
- Practical and effective protocols for emergency situations must be established. These protocols must include plans for medical emergencies, facility malfunctions, fires, escape of animals within the laboratory, and other potential emergencies. Training in emergency response procedures must be provided to emergency response personnel and other responsible staff according to institutional policies.

Table.1 : Relation of risk groups to biosafety levels, practices and equipment

Risk group	Biosafety level	Laboratory type	Laboratory practices	Safety equipment
1	Basic –Biosafety Level 1	Basic teaching, research	GMT	None; open bench work
2	Basic– Biosafety Level 2	Primary health services; diagnostic services, research	GMT plus protective clothing, biohazard sign	Open bench plus BSC for potential aerosols
3	Containment – Biosafety Level 3	Special diagnostic services, research	As Level 2 plus special clothing, controlled access, directional airflow	BSC and/or other primary devices for all activities
4	Maximum containment – Biosafety Level 4	Dangerous pathogen units	As Level 3 plus airlock entry, Shower exit, special waste disposal	Class III BSC, or positive pressure suits in conjunction with Class II BSCs, double ended autoclave (through the wall), filtered air

* GMT: good microbiological techniques

References:

1. Biosafety in Microbiological and Biomedical Laboratories 5th Edition
2. OIE *Terrestrial Manual* 2008

Agent Classification and Safety Levels

Jagadish Hiremath

Introduction:

The infectious organisms are categorized in to different risk groups based on the extent to which they can cause health risks on exposure. The characteristics like pathogenicity, infectious dose, transmission dose, host range, availability of effective vaccine and treatment will decide to which group the agent is categorized. The agents are grouped in to four risk groups (Risk group1to4) and this grouping is the basis for recommendation of Bio-safety levels (BSL1to 4).

Objectives:

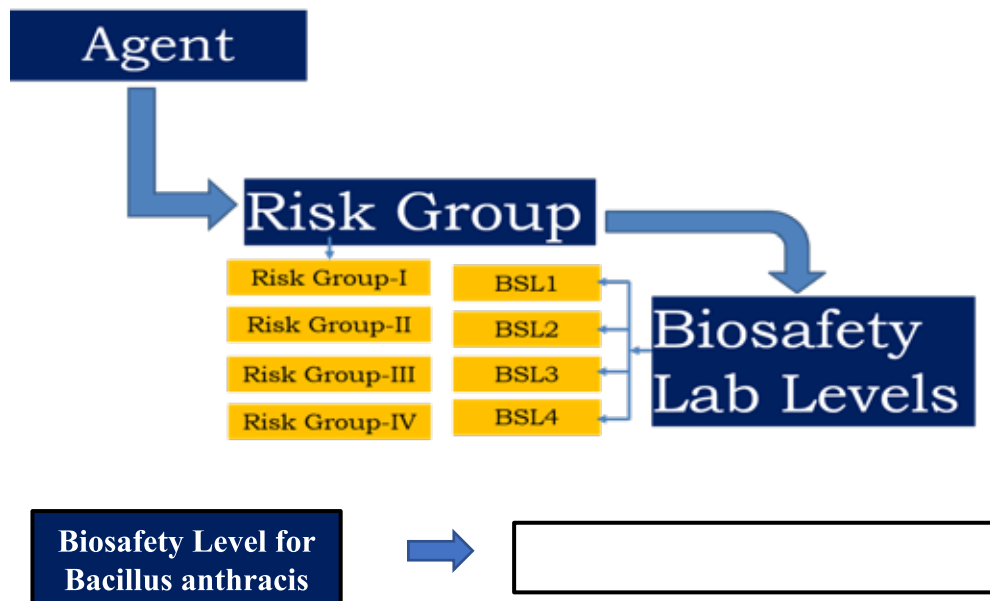
1. Demonstration of agent categorization
2. Identification of appropriate biosafety levels
3. Risk group categorization of agents using mobile applications

Activity:

1. Classification of *Bacillus anthracis* in to appropriate risk group using the risk group classification matrix provided below

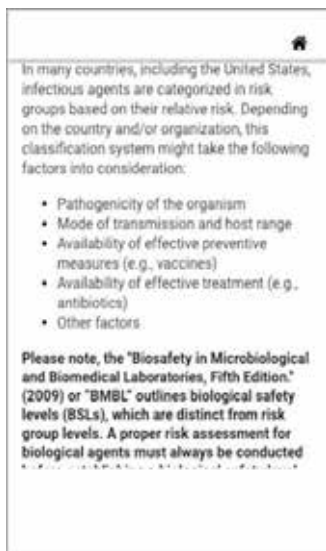
Disease in Human	Effective Prevention/Therapeutic	Single Host/Multi Host	Community risk	Risk Group

2. Identification of biosafety levels based on risk group categorization

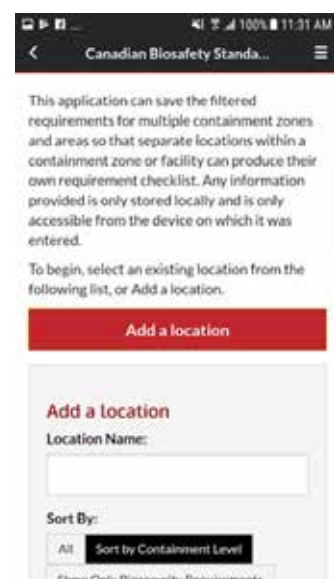
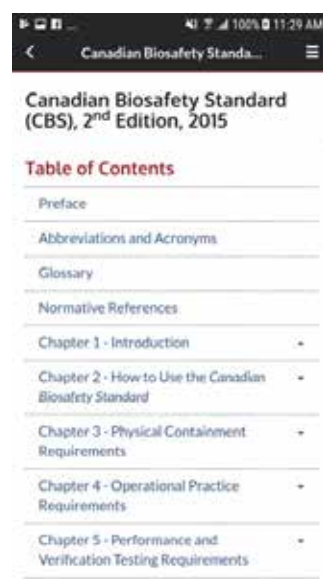


Risk Group Data Base (Mobile Application):

“Risk Group Data Base” is the mobile application developed by “American Biological Safety Association International” based on database of international risk group classification for various pathogens (bacteria, virus, fungi and parasites). The application categorizes the agents based on the Pathogenicity of the organism, Mode of transmission and host range, Availability of effective preventive measures (e.g., vaccines), Availability of effective treatment (e.g., antibiotics), and Other factors.



Canadian Biosafety Standards:



Indian BioSafety Application (IBSA):

Risk group categorization of biohazard agent is the basis for adopting appropriate Biosafety and biosecurity practices under laboratory and field conditions. Establishment of suitable containment facility is also based on the risk group of the agent. Towards this, the **IBSA** enables user to risk group categorization of biohazard agent of their interest. The operational guide for Biosafety laboratory recommended for a risk group with details of facility design, safety instruments, personal protective equipment, procedures, laboratory monitoring, waste management, health and medical surveillance and emergency procedures.



Mobile Screen



App Icon



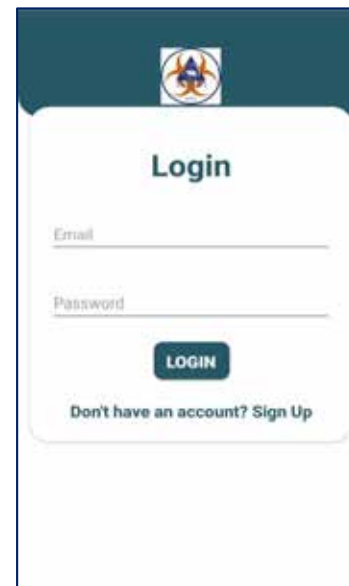
Welcome Logo Page



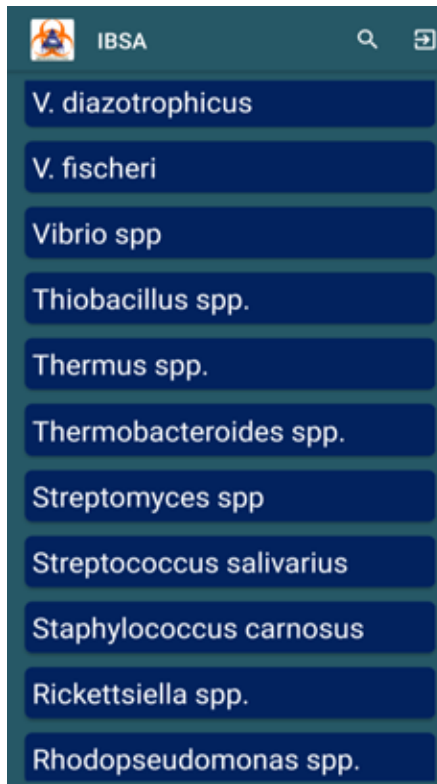
List of Risk Group Classification



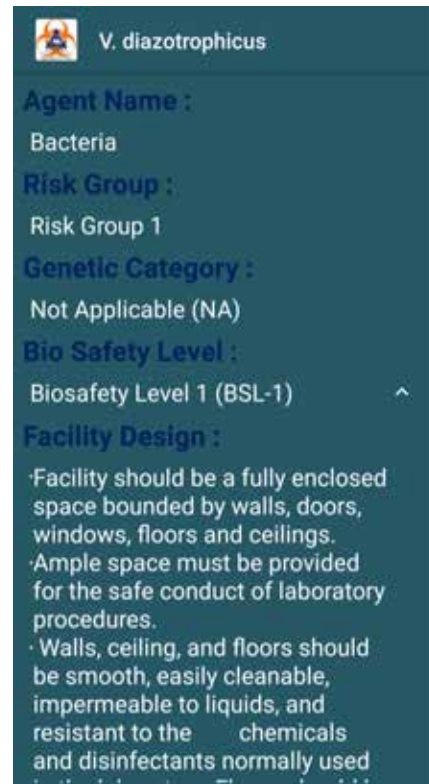
Home Page



Login/Sign Up Page



List of Species Categories



Species Categories Description

Exercise:

Using IBSA try identifying the risk group and biosafety levels of any two organism of your interest

5. National Biosafety Regulations in SAARC Member Countries

Jagadish Hiremath

ICAR-National Institute of Veterinary Epidemiology and Disease Informatics

Every country should have the guidelines to regulate the handling of hazardous microorganisms which will describe stringent and robust facility structures for handling of microorganisms, animals, plants, insects and aquatic organisms and should provide clear instruction on disposal and decontamination of laboratory wastes, emergency procedures etc. The guidelines should also provide the list of risk group agents and containment level required to handle them in a respective country. India has recently published a document “Regulations and Guidelines for Recombinant DNS research and Biocontainment 2017” which elaborately provides information regarding regulations and competent authorities, principles and components of containment, operational guides on containment and containment requirement for import, export and exchanges. Bangladesh has published the draft copy of Revised Biosafety Guidelines of Bangladesh 2018, under the scope of this document the biocontainment requirements, agent categorization, safety levels are all defined. Though most of the SAARC countries have national framework for handling GMO/LMO but the national guidelines for biocontainment are yet to be developed and implemented.

References:

1. Regulations and Guidelines for recombinant DNA research and bio containment 2017. Department of Biotechnology, Ministry of Science and Technology, Government of India (GOI).
2. Draft for amendment, Revised Biosafety Guidelines of Bangladesh 2018
3. Draft National Biosafety Framework of the Kingdom of Bhutan August 2006

6. Personal Protective Equipment (PPE)

R. Sridevi

ICAR-National Institute of Veterinary Epidemiology and Disease Informatics

Personal Protective Equipment commonly referred as PPE are those clothing's and equipment's worn to minimize exposure to serious workplace injuries and illnesses. PPE are used in different environment where the workplace includes chemical, radiological, physical, electrical, mechanical and biological hazards.

Personal protective Equipment (PPE) is a primary barrier which acts as a physical barrier between person and the materials with which he/she working with. Prevents spread of contamination and protect the product from contamination. PPE does not reduce or eliminate the hazard but only protects the wearer. There are different types of PPEs. Basically, classified into Hand protecting PPEs mainly Gloves(Latex Rubber, Nitrile, Rubber outer gloves), Face protecting PPEs which includes Goggles, Face Shield, Glasses, Splatter Guard, Respirator, Face mask, Complete body protecting PPEs which includes Lab coat, Full body coveralls, Fully encapsulated (supplied Air) and Foot covers or shoe covers or boot covers.

Usages of PPEs

Gloves are those equipments which cover the hands and wrists, protecting the skin from contact and droplet exposure. These are the most widely used type of PPE. Nitrile gloves have advantages of being allergies free and puncture resistant.

Goggles and glasses are used to protect the eyes from infectious droplets and from contact with infectious agents and accidental splashes. Goggles have advantageousness of fitting securely around the eyes than the glasses.

Face shields cover the face with a plastic shield and protects the face and the mucous membranes from infectious droplets and contact with contaminated materials.

Gowns protect person and personal clothing from infectious droplets and materials. Head covers protect the spread of infectious particles through droplet contact. Masks protect the personal from airborne and droplet contaminants. There are different types of masks simple surgical mask to Special masks called as N95, N99 or N100 Respirators which are worn specifically for highly infectious particles.

Protective laboratory clothing – Lab coats, gowns or uniforms be worn to prevent contamination or soiling of street clothes in BSL-1. These are must while working in BSL2 facilities and should be removed and left in laboratory before leaving for non-laboratory areas. Reusable clothings should be placed in a closed container and subsequently sterilized before laundering. Labcoats must be worn all the time in laboratory.

Protective eyewear should be worn during procedures in which splashes of microbes or other hazardous materials possible.

Face protection (goggles, mask, face shield or splatter guard) are used when accidental splashes or sprays of infectious materials and other hazardous materials expected.

Gloves must be worn when handling BSL-1/BSL-2/BSL-3 materials or when coming into contact with potentially contaminated surfaces or equipment. Wearing double pairs of gloves is appropriate while handling zoonotic agents. While handling chemical hazards, Material Safety

Data Sheet (MSDS) should be consulted. Gloves should be disposed after working with infectious materials and should not be reused or washed or should not be used in clean areas. Hands should be washed immediately following gloves removal.

Footwears –closed –toed shoes with backs are used. And in infected animal handling areas tight boots are preferred.

Personal protective equipments vary depending upon the biological safety level. The following table depicts PPE Requirements based on Biological Safety levels

PPE Requirements for various Biological safety levels			
BSL-1	BSL-2	BSL-3	BSL-4
PPEs -Labcoats -Safety glasses/ Goggles -Gloves (People wearing contact lenses should wear eye protection)	PPEs -Long sleeved and buttoned Laboratory coats/ gowns,smocks,or uniforms -Goggles,face mask,face shield -Gloves -Eye,face and respiratory protection should be used in rooms containing infected animals. -Footwears- lab wears	PPEs -Lab clothing with solid-front,-wrap- around gowns, scrub suits with tight cuffs. -Goggles,mask,face shield or other splash guards along with BSC -Gloves –single/ double Eye, face and respiratory protection should be used in rooms containing infected animals Other PPE based on risk assessment * Coveralls * Booties, * Head Covers * Double Gloves * Respirators * Disposable sleeves * Footwears /shoe- covers	Separate Lab wears Positive pressure Suits A Chemical shower upon exit for decontamination of positive pressure suits.

Finally, Lab personnel/In-charge working should always ensure i) availability of appropriate PPE intended for work ii) proper training of personnel working inside labs. Iii) Proper usage of PPE and iv) Appropriate disposal of PPEs.

References:

1. Arizona State University Fact Sheet on Personal Protective Equipment Requirements for work with Biological materials. EH&S. December, 2016.

2. <http://www.cdc.gov/OD/biosfty>

Practical

Personal Protective Equipment (PPE)

Jagadish Hiremath

Introduction:

Personal protective equipment is specialized clothing or equipment designed to be worn by some one to protect themselves against possible exposure to biohazard agents either in laboratory or in field settings. There are different types of PPE recommended for each type of biosafety level. Balancing the levels of PPE usage in order to achieve the optimum levels of protection while keeping comfortable to work is important. Too much of PPE or too less of PPE both will have bearing on the work practices and/or safety of an individual.

Objectives:

1. To discuss and demonstrate the basis for PPE selection
2. To demonstrate the sequence of donning and removing PPE
3. To demonstrate the safe disposal procedures of PPE

Activity:

1. The types of PPE and basis for PPE selection will be shown to the trainees (Fig.1)



Fig.1 PPE for protection against A. Cutaneous and B. Respiratory route of infection

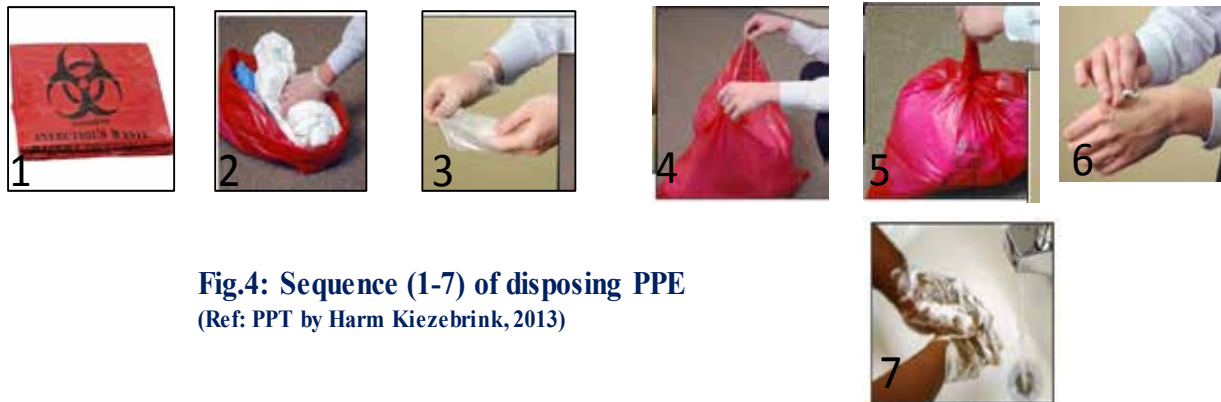
2. The sequence of donning PPE will be demonstrated with help of trainees (Fig.2)
3. The sequence of removing the PPE will be demonstrated with help of the trainees (Fig.3)
4. The demonstration of safe disposal of PPE (Fig.4)



Fig.2: Sequence of donning PPE



Fig.3: Sequence of removing PPE



7. Signages










Jagadish Hiremath

ICAR-National Institute of Veterinary Epidemiology and Disease Informatics

Signages in biosafety laboratory are designed to alert the laboratory personnel, staff, students, visitors and emergency responders to specific hazards located in individual laboratories. The biosafety induction training for the personnel working in biosafety laboratory should cover the important signalization symbols and their meaning. Understanding the signages and their display at laboratory entry, inside laboratories, on the storage equipment and other relevant areas is a part of good laboratory practices. List of signages along with description is provided in the table below

	Biohazard sign
	No sharps Sign
	Do not turn off Sign
	Do not touch Sign
	No drinking Sign
	No eating Sign
	No food or drink Sign

	<p>Strictly no admittance Sign</p>
	<p>No naked flames Sign</p>
	<p>No smoking Sign</p>
	<p>No exit Sign</p>
	<p>Not drinking water Sign</p>
	<p>No sharp objects Sign</p>
	<p>Gloves Required</p>
	<p>High Voltage</p>
	<p>Hot Surface</p>

	Low Temperature
	Flammable
	Harmful
	Electrical Hazard
	Toxic Material Hazard
	Ionizing Radiation Hazard
	Corrosive Material Hazard
	Glassware Hazards
	Carcinogen Hazard

DANGER
 RESTRICTED AREA
 AUTHORIZED
 PERSONNEL
 ONLY



CAUTION

Minimum Personal Protective Equipment Requirements:

No eating, drinking or smoking in this area

Additional PPE requirements may be needed while working in the lab, contact your supervisor.

Special Hazards or Condition: **BSL: 2**

Primary Lab Contact: Karen Chen 6297
 Secondary Lab Contact: Steve Sadek 6296

For an Emergency (Fire, Life-Threatening), Dial 9 911
 For Internal Help, Dial 5911
 For EMS Dept., 6207

Biosafety Laboratory (BSL) 2++
 ICAR-National Institute of Veterinary Epidemiology and Disease Informatics
 Yashwantrao Chavan Marg, Solapur-431004

Safety measures in place

Fire extinguisher, First Aid kit, Eye wash, Safety shower, CCTV, BSC, Negative Pressure Cabinet, LAF C, SSS, ESD, Fire alarm, Exit, Cameras, Biohazard Waste, Corrosive.

Identified Hazards

BIOLOGICAL HAZARD, BIOHAZARD WASTE, Corrosive.

Entry Requirements / **Protective Measures Required**

NO ENTRY (AUTHORIZED PERSONS ONLY), NO FOOD OR DRINK, Lab coat, Goggles.

Contact details:

Sl. No.	Name	Designation	Contact number/Email
01	Dr. Jagadik Haranath	Biosafety Officer	+91 90 23003142 jagiret@gmail.com
02	Dr. R. Suresh	Asst. Biosafety Officer	+91 90 23003144 rsharanjag@gmail.com
03	Dr. G.B. Manjunatha Reddy	Asst. Biosafety Officer	+91 90 23003145 gbsrath@icar.com
04	Dr. Yashwantrao	Asst. Biosafety Officer	+91 90 23003151 dr.yashwantrao@gmail.com

BIOHAZARD

ANIMAL BIOSAFETY LEVEL 2

Principal Investigator: Sonbir Lee
 Agent (D): Manohararam Jetti (MCG/Animal)
 Bld: G/BBB Room: 15923B

Requirements for Laboratory Entry:

- Proper GERM suppression training.
- Compliance with PPE (lab) was required prior to work with the materials.
- PPE: lab coat, shoe cover, mask, hood, full face protection, safety glasses (worn tied to forehead), cover mouth, hood, full face protection.
- Use another pair of pair of booties while in the laboratory leaving the lab.
- Hand: A biohazard PAPER is essential. It must be held like the photo of mask, hood and full-face protection.

Leave Lab:

- Remove outer gloves, apron, hood, and cover booties while in the laboratory.
- Remove all other PPE in the laboratory.
- Wash hands with soap and water.

EMERGENCY SERVICE	CONTACT	PHONE NUMBER	CELL NUMBER
Fire	Fire Dept.	911/022	911/022
Police	Police Dept.	100/022	100/022
Medical	Medical Dept.	102/022	102/022
Transport	Transport Dept.	101/022	101/022

Introduction:

Signages in biosafety laboratory are designed to alert the laboratory personnel, staff, students, visitors and emergency responders to specific hazards located in individual laboratories. The biosafety induction training for the personnel working in biosafety laboratory should cover the important signalization symbols and their meaning. Understanding the signages and their display at laboratory entry, inside laboratories, on the storage equipment and other relevant areas is a part of good laboratory practices.

Objectives:

1. To demonstrate the signages that should be placed in each room entrance, in each laboratory room and on equipment
2. To demonstrate custom designing of signages using online software “ Safety Sign Builder”

Activity:

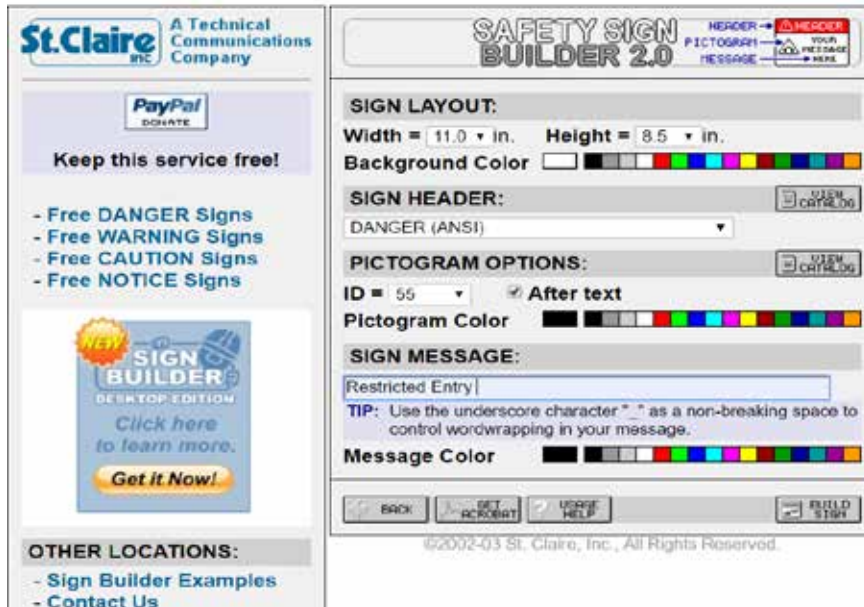
1. The trainees are taken to different areas of the laboratory and discussed regarding signages to be displayed in each identified areas
2. Demonstration of preparation of signages with Header, Pictogram and Message using the online software “Safety Sign Builder 2.0”
 - i. Use the following web address to reach “ Safety Sign Builder 2.0” home page

http://www.stclair.com/safety_sign_builder/ssb-panel.php

- ii. Register using link for new user to receive User ID and Password
- iii. Pay attention towards **Sign Layout, Sign Header, Pictogram Option and Sign Message**



- iv. Sign lay out has option of **size** (Width & Height) and **background color**. Similarly sign header has options of choosing type signage you would like to have (View Catalogue for the options), **Pictogram options** will provide you more than 200 signages (View catalogue and pay attention to their ID for choosing appropriate signages) and you have option to choose the position of the signage and color. **Sign Message** the last feature has scope for customizing your message and option to choose message color



Input:

Sign Layout: Width-11.00, Height-6.5, Background color-White

Sign Header: Danger

Pictogram Options: ID-55, After Text, Color- Black

Sign Message: Restricted Entry, Color- Black

- V. The output signage in PDF format



Exercise:

Prepare at least one each signage to display on the entrance of the laboratory and inside

8. Disinfection & Antiseptics

R. Sridevi

ICAR-National Institute of Veterinary Epidemiology and Disease Informatics

Decontamination is any activity that reduces the microbial contamination of materials or surfaces to prevent inadvertent infection. Disinfection refers to the elimination of virtually all pathogenic organisms on inanimate objects and surfaces thereby reducing the level of microbial contamination to an acceptably safe level. Sterilization refers to the destruction of all microbial life, including bacterial endospores. When choosing a disinfectant one should consider the organism, the item to be disinfected, and the cost and ease of use of the disinfectant. An antiseptic is a substance that stops or slows down the growth of microorganisms. Here are some of the commonly used Disinfectants of Biosafety Laboratory given in detail, but depending upon the organisms handled and facilities available the disinfectants should be chosen and used.

Bleach - Chlorine (Sodium Hypochlorite)

Chlorine, a fast-acting oxidant, is a widely available and broad-spectrum chemical disinfectant. It is normally sold as household bleach, an aqueous solution of sodium hypochlorite (NaOCl), which can be diluted with water to provide various concentrations of available chlorine. Chlorine is highly alkaline and can be corrosive to metal. The disinfectant activity of chlorine is considerably reduced by organic matter (protein). Storage of stock or working solutions of bleach in open containers, particularly at high temperatures, may release chlorine gas thus weakening their disinfectant potential. **Undiluted household bleach stored at room temperature in the original container has a shelf-life of approximately one year.** Working solutions of bleach should be prepared on a daily basis. Household bleach (typically 5.25% NaOCl, check the label) should be diluted 1:10 to obtain final concentration of 0.5% NaOCl. Industrial solutions of bleach have a higher sodium hypochlorite concentration and must be diluted accordingly to obtain the correct concentration. To increase the efficacy of sodium hypochlorite solutions against spores, vinegar may be added to the solution. Combine 5 ounces of household bleach with one gallon of water and add 8 ounces of 5% distilled white cooking vinegar to the diluted bleach solution. Chlorine gas is highly toxic. Bleach must therefore be stored and used in well-ventilated areas only. Undiluted bleach must not be mixed with acids or other incompatible chemicals, such as ammonia containing compounds, to prevent the rapid release of chlorine gas. Many by-products of chlorine can be harmful to persons.

Chlorhexidine also known as chlorhexidine gluconate (CHG), is a disinfectant and antiseptic that is used for skin disinfection before surgery and to sterilize surgical instruments. It may be used both to disinfect the skin of the patient and the hands of the healthcare providers. It is also used for cleaning wounds, preventing dental plaque, treating yeast infections of the mouth, and to keep urinary catheters from blocking. It is used as a liquid or powder. It is a cationic polybiguanide (bisbiguanide) and primarily used as its salts (eg: dihydrochloride, diacetate, digluconate). Chlorhexidine is deactivated by forming insoluble salts with anionic compounds, Chlorhexidine is used in disinfectants (disinfection of the skin and hands). It is effective against wide range of microbes but does not inactivate spores. Side effects may include skin irritation, teeth discoloration, allergic reactions and eye problems if direct contact occurs. At physiologic pH, chlorhexidine salts dissociate and release the positively charged chlorhexidine cation. The bactericidal effect

is a result of the binding of this cationic molecule to negatively charged bacterial cell walls. At low concentrations of chlorhexidine, this results in a bacteriostatic effect; at high concentrations, membrane disruption results in cell death.

Alcohols

Ethanol (ethyl alcohol, C₂H₅OH) and 2-propanol (isopropyl alcohol, (CH₃)₂CHOH) have similar disinfectant properties. They are active against vegetative bacteria, fungi, and lipid-containing viruses but not against spores. Their action on non-lipid-containing viruses is variable. For highest effectiveness they should be used at concentrations of approximately 70% (v/v) in water: higher or lower concentrations may not be as germicidal. A major advantage of aqueous solutions of alcohols is that they do not leave any residue on treated items. Mixtures with other agents are more effective than alcohol alone, for example 70% (v/v) alcohol with 100 g/L formaldehyde and alcohol containing 2 g/L available chlorine. A 70% (v/v) aqueous solution of ethanol can be used to soak small pieces of surgical instruments. A contact time of ten minutes or more is necessary. Ethanol should never be used to disinfect hands since ethanol can dry the skin. Alcohol-based hand-rubs, alcohol mixed with emollients, are recommended for the decontamination of lightly soiled hands in situations where proper hand-washing is inconvenient or not possible. However, it must be remembered that ethanol is ineffective against spores, HBV, Mycobacterium tuberculosis (TB) and may not kill all types of non-lipid-containing viruses. Alcohols are volatile and flammable and must not be used near open flames. **Do not use 70% ethanol to clean a Class II, Type A recirculating biosafety cabinet.** The vapors from ethanol are flammable and the lower explosive limit (LEL) for ethanol is easily attained. Working solutions should be stored in proper containers to avoid the evaporation of alcohols. Alcohols may harden rubber and dissolve certain types of glue. **Proper inventory and storage of ethanol in the laboratory is very important to avoid its use for purposes other than disinfection.** Bottles with alcohol-containing solutions must be clearly labeled to avoid autoclaving.

References:

1. World Health Organization Laboratory Biosafety Manual,
<http://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf>
2. EPA Approved Disinfectants Website,
<http://www.epa.gov/oppad001/chemregindex.htm>

Practical

Laboratory Cleaning and Hand Washing

Jagdish Hiremath

Introduction:

The laboratory must be clean and tidy. This decreases the risk of accidents and laboratory associated infections. Moreover, a clean environment also decreases the risk of negative influences on sensitive examinations (such as culture or PCR) through contamination. Set up a cleaning schedule. The laboratory building must be regularly cleaned by dedicated and trained cleaning staff. A cleaning record has to be maintained in each laboratory depicting the date, time, sign of the cleaning staff and sign of the verifier. A standard operating procedure for laboratory cleaning should be prepared and documented as this is an

imported requirement for establishing good house keeping practices.

Hand washing is an important and effective biosafety practice to prevent the exposure of infection or transmission of the same from inside of the laboratory to outside. World health organization has recommended 10 step hand washing which spans for 40-60sec. A proper hand wash includes following steps

- ❖ Wet your hands with warm, running water and apply liquid soap or use clean bar soap. Lather well.
- ❖ Rub your hands vigorously together for at least 15 to 20 seconds.
- ❖ Scrub all surfaces, including the backs of your hands, wrists, between your fingers and under your fingernails.
- ❖ Rinse well.
- ❖ Dry your hands with a clean or disposable towel.
- ❖ Use a towel to turn off the faucet.

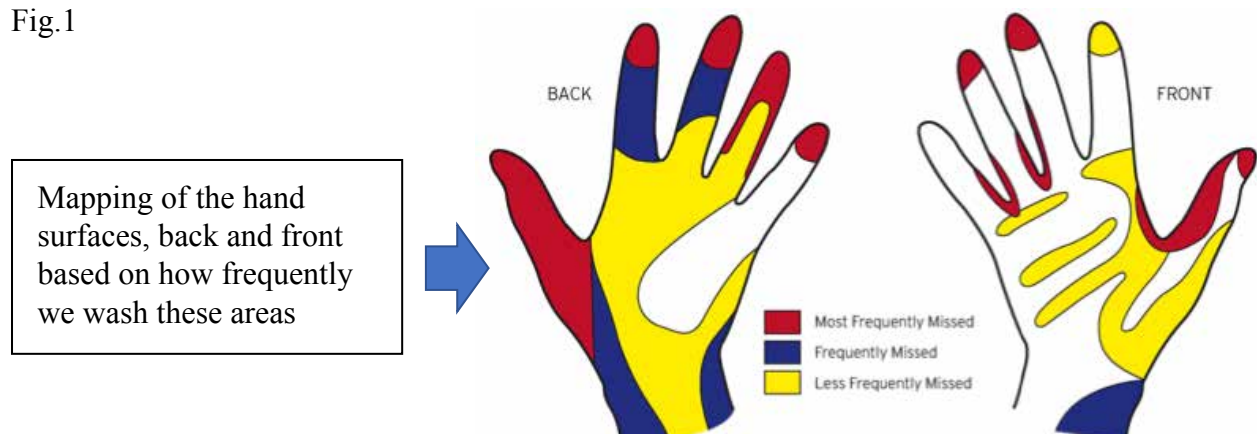
Objectives:

1. To demonstrate the laboratory cleaning steps
2. To demonstrate the proper handwashing techniques

Activity:

1. The laboratory cleaning practices followed in BSL2 laboratory and documentation to be maintained (SOP, cleaning log) for routine cleaning procedure.
2. Trainees will wash their hands adopting proper hand washing techniques as shown in

Fig.1



How to Handwash?

WASH HANDS WHEN VISIBLY SOILED! OTHERWISE, USE HANDRUB

 **Duration of the entire procedure: 40-60 seconds**



0 Wet hands with water;



1 Apply enough soap to cover all hand surfaces;



2 Rub hands palm to palm;



3 Right palm over left dorsum with interlaced fingers and vice versa;



4 Palm to palm with fingers interlaced;



5 Backs of fingers to opposing palms with fingers interlocked;



6 Rotational rubbing of left thumb clasped in right palm and vice versa;



7 Rotational rubbing, backwards and forwards with clasped fingers of right hand in left palm and vice versa;



8 Rinse hands with water;



9 Dry hands thoroughly with a single use towel;



10 Use towel to turn off faucet;



11 Your hands are now safe.



World Health Organization

Patient Safety

A World Alliance for Safer Health Care

SAVE LIVES

Clean Your Hands

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May 2009

9. Biosafety Cabinets: Types, Practices and Safe Behaviours

Manjunatha Reddy, G.B and Jagadish Hiremath

ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI)

Biological agents need to be managed in the laboratory environment so as to prevent / control the exposure of laboratory workers, other people and the outside environment to these agents. One of the primary containment devices used is biosafety cabinet or biological safety cabinet (BSC) or microbiological safety cabinet or laminar flow cabinets. All exhaust air from BSC is HEPA-filtered as it exits the cabinet, removing harmful bacteria and viruses, which is in contrast to a laminar flow clean bench, which blows unfiltered exhaust air towards the user and is not safe for working with pathogenic agents. In a similar way the fume hood fails to provide the environmental protection that HEPA filtration in a BSC would provide. Along with primary purpose of filtering, most classes of BSCs have a secondary purpose to maintain the sterility of materials inside. Therefore a properly functioning BSC is essential for working with infectious agents in any laboratory.

Classes of Biosafety Cabinets

1. Class I Biosafety Cabinets
2. Class II Biosafety Cabinets
3. Class II Type A (A1/A2) Biosafety Cabinets
4. Class II Type B Biosafety Cabinets
5. Class II Type B1 Biosafety Cabinets
6. Class II Type B2 Biosafety Cabinets
7. Class III Biosafety Cabinets

Class	Work Opening	Inflow Velocity (fpm)	% Recirculated Air	% Exhausted Air	Exhaust Volume (cfm)	Exhaust Requirement	Application
I	Fixed	75	0%	100%	4 ft - 200 6 ft - 300	Exhausted to the outside or to the room through a HEPA filter	Biosafety Level 1-3
II, type A1	fixed, sliding, or hinged	75-100	70%	30%	4 ft - 300 6 ft - 400	Exhausted to room through HEPA filter or to the environment through an exhaust canopy	Biosafety Level 1-3
II, type B1	Sliding	100	30%	70%	4 ft - 250 6 ft - 400	Exhausted to outside, with remote fan; duct is hard connected	Biosafety Level 1-3
II, type B2	sliding, hinged	100	0%	100%	4 ft - 600 6 ft - 1000	Exhausted to outside, with remote fan; duct is hard connected	Biosafety Level 1-3
II, type A2	sliding, hinged	100	70%	30%	4 ft - 300 6 ft - 400	Exhausted to room through HEPA filter or exhausted to outside, with remote fan, utilizing canopy connection	Biosafety Level 1-3
III	Glove ports	N/A	0%	100%	Refer separately	Exhausted to outside, through 2 HEPA filters, with remote fan; duct is hard connected	Biosafety Level 1-4

Source: <https://unc.policystat.com/policy/5819516/latest/>

Filters of biosafety cabinets

The HEPA (High Efficiency Particulate Air) / ULPA (Ultra Low Penetration Air) filter is the heart of the biological safety cabinet. The HEPA filter is a disposable dry-type filter, constructed of borosilicate microfibers cast into a thin sheet, much like a piece of paper. The difference between HEPA and ULPA filter is size of 0.3 microns and 0.12 microns respectively. The HEPA/ ULPA filters are designed to remove a broad range of airborne contaminants, including: fine dust, smoke, bacteria, viruses, soot, pollen, radioactive particles and impurity ions.

Certification of biosafety cabinets

The certification from any of the following international standards agency are recommended for BSC's:

- American Standard NSF 49 (National sanitation foundation)
- European Standard EN 12469
- Australian Standard AS 2252
- Japanese Standard JIS K 3800

The standard certification agency compliance includes but not restricted to requirements for design, construction and performance in order to provide personnel, product and environmental protection, reliable operation, and durability as well as being easy to clean. In addition to above onsite certifiers/calibration is recommended at regular time interval during which the following tests are performed.

- ✓ Down-flow velocity test
- ✓ Inflow velocity test
- ✓ Airflow Smoke Patterns test
- ✓ HEPA filter test
- ✓ Cabinet integrity test (A1 cabinets only):
- ✓ Site installation integrity tests

Selection of biosafety cabinet

Any BSC selection is primarily depends on type of protection needed, product protection, personnel protection against Risk Group of microorganisms, personnel protection against exposure to radionuclides and volatile toxic chemicals; or a combination of these. The following table gives details about type of BSCs recommended for each type of protection.

Type of protection	BSC Selection
Personnel Protection, microorganisms in Risk Groups 1-3	Class I, Class II, Class III
Personnel Protection, microorganisms in Risk Groups 4, glove box laboratory	Class III
Personnel Protection, microorganisms in Risk Groups 4, suit laboratory	Class I, Class II
Product Protection	Class II, Class III only if laminar flow included

Type of protection	BSC Selection
Volatile radionuclide / chemical protection, minute amounts	Class II Type B1, Class II Type A2 vented to the outside
Volatile radionuclide / chemical protection	Class I, Class II Type B2, Class III

Safe use of biosafety cabinets in the laboratory

The following points should be given consideration while using the biosafety cabinet in laboratory. For more details visit <https://www.cdc.gov/labs/BMBL.html>.

1. Location
2. Operators
3. Material Placement
4. Operation and Maintenance
5. Ultraviolet Lights
6. Open flames
7. Spills
8. Annual certification
9. Cleaning and Disinfection
10. Decontamination
11. PPE
12. Alarms

To conclude any person working with biohazards materials should know the importance BSCs to ensure proper functioning to avoid the consequences of using faulty equipment which can range from contaminated product and wasted research time to serious illness and/or death of laboratory personnel.

Practical

Maintenance of BioSafety Cabinet (BSC)

Jagadish Hiremath

Introduction:

Biosafety cabinets (BSC) are critical safety equipment that are widely used as primary barrier that provided personnel or both personal and product protection from biohazardous material. BSC are equipped with High Efficiency Particulate Air (HEPA) filter that has efficiency of 99.99% at 0.3microns, HEPA filters are disposable dry-type filter, constructed of borosilicate microfibers cast into a thin sheet, much like a piece of paper.

Objectives:

1. To demonstrate the types of biosafety cabinets and their working principle
2. To demonstrate the best practices while working with BSC
3. To demonstrate the validation tests of BSC

Activity:

1. Types of cabinet: Class-I, Class-II (A1, A2, B1, B2), Class-III
2. Video showing the best practices while working with BSC will be screened and discussed
3. Tests used for validating the performance of BSC (Video Screening)

10. Emergencies in biosafety lab and ways to handle them

Jagadish Hiremath

ICAR-National Institute of Veterinary Epidemiology and Disease Informatics

Laboratory emergencies are serious, unexpected and sometime even dangerous situation requiring immediate action to prevent worsening of the situation. The most common laboratory emergencies include biological or chemical spillages. The fire and electrical emergencies are also often reported. Most laboratory accidents occur due to poor planning and deviating from good laboratory practices. As popularly said “prevention is better than cure” it is always better to prevent the accidents than working during laboratory accidents. During emergency it is essential to ensure the personal safety first and then call for local responders if necessary. Alerting the other laboratory personnel regarding the accident is necessary for their protection. The extent of response will be based on the seriousness of the incident and preparedness to handle it. Stay calm and take proper actions according to the type and level of emergency.

A. Biological Spillage

Major Spills

- Attend to injured or contaminated persons and remove them from exposure.
- Alert people in immediate area of spill, if need be evacuate from the place.
- Close doors and mark the area as “Biological spill” in the area.
- Put on PPE.
- Cover spill absorbent material like paper towel disposal mops etc.,
- Pour 1% to 2% freshly prepared bleach around the edges of the spill and then into the spill. Avoid splashing.
- Allow at least 20-minute contact period for the bleach solution to be effective.
- Use paper towels to wipe up the spill, working from the edges into the centre.
- Clean spill area with fresh towels soaked in disinfectant.
- Place towels in a plastic bag and decontaminate in an autoclave.
- Have person knowledgeable of the incident and laboratory assist emergency personnel.

Minor

- Wear disposable gloves.
- Soak paper towels in disinfectant and place over spill area.
- Place towels in plastic bag for disposal.
- Clean spill area with fresh towels soaked in disinfectant.

Note:

1. Biological spills outside biological safety cabinets (BSC) will generate aerosols that can be dispersed in the air throughout the laboratory. These spills are very serious if they involve microorganisms that require Group 3 containment, as most of these agents have the potential for transmitting disease by infectious aerosols.
2. To reduce the risk of inhalation exposure in such an incident, occupants should hold their breath and leave the laboratory immediately. The laboratory should not be re-entered to decontaminate and cleanup the spill for at least 30 minutes. During this time the aerosol will be removed from the laboratory by the exhaust air ventilation system.

3. Appropriate protective equipment is particularly important in decontaminating spills involving microorganisms. This equipment includes lab coat with long sleeves, back-fastening gown, disposable gloves, disposable shoe covers, and safety goggles and mask or full-face shield. Use of this equipment will prevent contact with contaminated surfaces and protect eyes and mucous membranes from exposure to splattered materials.

(Ref: <http://www.safety.uwa.edu.au/incidents-injuries-emergency/procedures/lab#biological-spill>)

B. Chemical Spillage

Major Spills

- Attend to injured or contaminated persons and remove them from exposure.
- Alert people in the laboratory to evacuate.
- If spilled material is flammable, turn off ignition and heat sources.
- Call for assistance.
- Close doors to affected area.
- Have person knowledgeable of incident and laboratory assist emergency personnel

Minor Spills

- Alert people in immediate area of spill.
- Wear protective equipment, including safety goggles, gloves, and long-sleeve laboratory coat.
- Avoid breathing vapors from spill.
- Confine spill to small area.
- Use appropriate kit to neutralise and absorb inorganic acids and bases. Collect residue, place in container, and dispose as chemical waste.
- For other chemicals, use appropriate kit or absorb spill with vermiculite, dry sand, or diatomaceous earth. Collect residue, place in container and dispose as chemical waste.

Note:

- a) The range and quantity of hazardous substances used in laboratories require preplanning to respond safely to chemical spills.
- b) The cleanup of a chemical spill should only be done by knowledgeable and experienced personnel.
- c) Spill kits with instructions, absorbents, reactants, and protective equipment should be available to clean up minor spills.
- d) A minor chemical spill is one that the laboratory staff is capable of handling safely without the assistance of safety and emergency personnel. All other chemical spills are considered major.

(Source: <http://www.safety.uwa.edu.au/incidents-injuries-emergency/procedures/lab#chemical>)

C. Biological Spill on Body

Body other than eyes:

- Remove contaminated clothing.

- Vigorously wash exposed area with soap and water for one minute.
- Obtain medical attention, if necessary.
- Report incident to supervisor.

Eyes:

- Immediately rinse eyeball and inner surface of eyelid with water continuously for 15 minutes.
- Forcibly hold eye open to ensure effective wash behind eyelids.
- Obtain medical attention.
- Report incident to supervisor.

(Source: <http://www.safety.uwa.edu.au/incidents-injuries-emergency/procedures>)

D. Chemical Spill on Body

- Flood exposed area with running water from faucet or safety shower for at least 5 minutes.
- Remove contaminated clothing at once.
- Make sure chemical has not accumulated in shoes.
- Obtain medical attention, if necessary.
- Report incident to supervisor.

(Source: <http://www.safety.uwa.edu.au/incidents-injuries-emergency/procedures>)

E. Fire Emergency

Major

- Alert people in area to evacuate.
- Activate nearest fire alarm or call Security number.
- Close doors to confine fire.
- Evacuate to safe area or exit building through stairwell; do not use lift.
- Have person knowledgeable of incident and laboratory assist emergency personnel.

Minor

- Alert people in laboratory and activate alarm.
- Smother fire or use correct fire extinguisher.
- Aim extinguisher at base of fire.
- Always maintain accessible exit.
- Avoid smoke or fumes

(Source: <http://www.safety.uwa.edu.au/incidents-injuries-emergency/procedures>)

Note:

1. Small fires can be extinguished without evacuation.
2. Fire extinguishers should only be used by trained personnel.
3. Never enter a room that is smoke filled.
4. Never enter a room containing a fire without a backup person.

(Ref. <http://www.safety.uwa.edu.au/incidents-injuries-emergency/procedures>)

Emergencies in Biosafety Labs: Biological Spillage

Jagadish Hiremath

Introduction:

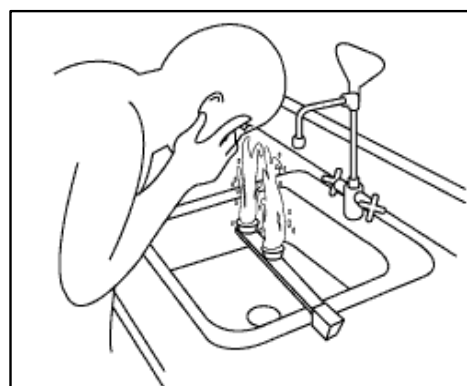
Laboratory emergencies are serious, unexpected and sometime even dangerous situation requiring immediate action to prevent worsening of the situation. The most common laboratory emergencies include biological or chemical spillages.

Objectives:

1. To demonstrate the use of eye washer during spillages
2. To demonstrate the procedures to cleanup biological spills outside of BSC
3. To demonstrate the procedure to cleanup biological spills inside the BSC

Activity:

1. **Use of eye washer during spillage:** Eye washer is the requirement in BSL2 laboratory and this can be used in accidental biological or chemical spills in to the eyes.



2. Demonstration of procedures to cleanup biological spills inside of BSC

- Don't turn off the BSC and wait for minimum of 5minute before beginning cleanup procedure.
- Use PPE (lab coat, safety glasses and gloves) during cleanup.
- Apply disinfectant (1% to 2% Bleach) starting from periphery to centre and allow a minimum of 20 minutes contact time.
- Wipe up spillage with disposable disinfectant-soaked paper towel.
- Wipe the walls, work surface and any equipment in the cabinet with a disinfectant soaked paper towe.
- Discard contaminated disposable materials using appropriate biohazardous waste disposal procedures (e.g. autoclave).
- Place contaminated reusable items in biohazard bags, autoclavable pans with lids or wrap in newspaper before autoclaving and cleanup.
- Expose non-autoclavable materials to disinfectant (20 minute contact time) before removal from the BSC.

- Remove protective clothing used during cleanup and place in a biohazard bag for autoclaving.
- Run cabinet 10 minutes after cleanup before resuming work or turning cabinet off.

3. Demonstration of procedures to cleanup biological spills outside of BSC

- Call the biosafety office if the material is BSL-2 or greater containment.
- Clear area of all personnel. Wait at least 15 minutes for aerosol to settle before entering spill area.
- Remove any contaminated clothing and place in biohazard bag to be autoclaved.
- Put on a disposable gown, safety glasses and gloves.

Spill Cleanup Procedures

Absorbent Pad Spill Kit



- ① Place a barrier around the spill
- ② Cover completely with appropriate material
- ③ Clean up
- ④ Bag and tag for EH&S waste removal

Floor Dry Spill Kit



IOWA STATE UNIVERSITY
OF SCIENCE AND TECHNOLOGY

11. Laboratory waste management: Autoclave and ETP

M. Nagalingam & R. Sridevi

ICAR-National Institute of Veterinary Epidemiology and Disease Informatics

Laboratory waste management is fundamental for maintaining the laboratory and the environment clean and healthy. In laboratories, decontamination of wastes and their ultimate disposal are closely interrelated. According to WHO, health care waste are categorized in to sharps waste, infectious waste, pathological waste, pharmaceutical waste, cytotoxic waste, chemical waste, radioactive waste and non hazardous or regular health care waste. All the above wastes are generated in the laboratories and each waste has their specific way of disposal. In microbiology laboratories, the generation of infectious waste is of prime importance and its management is detailed here. The infectious waste can be of two types.

i. Solid waste

Waste generated from disposable items other than sharp items.

Eg. Disposable culture plates, pipette tips etc.,

ii. Liquid waste

Waste generated from laboratory and washing, cleaning, house-keeping and disinfecting activities

Eg. Liquid discard during DNA extraction

Generally, Solid wastes are disinfected by autoclaving while liquid wastes are disinfected by chemical treatment and discharged into drains, but autoclaving is also practised. The drain waste water is subjected to Effluent treatment plant (ETP).

Collection of wastes

Liquid waste does not require bags. Sharp wastes are disposed at specific sharp waste containers kept at the generation point. Solid wastes are collected in leak-resistant heavy duty bags colour coded with appropriate signs according to the category and should have labels mentioning date and details of waste. The bags to be tied tightly after they are three- fourth full. Each laboratory should have waste management plan which should include collection points and routes of waste transport. A time table for picking up the waste should be determined and usually no untreated biomedical waste should be kept or stored beyond a period of 48 hrs.

Decontamination

Steam autoclaving is the preferred method for all decontamination processes. Autoclaving works on the principle of direct contact of pressurized steam in a controlled manner and for sufficient duration. The autoclave should be dedicated for the purposes of disinfecting and treating laboratory waste.

Autoclaving types

Horizontal autoclaves are suitable for handling lighter loads and reduce strain for the handlers where as vertical autoclaves make the process easier, safer, more precise, reproducible and easier to validate. When safety and contamination are a concern, a pass-through autoclave is a must

which will have two doors one at the internal side of the laboratory and other at the external side. Autoclaves function primarily through either gravity or vacuum-induced or pre-vacuum (prevac) sterilization methods, though some types of autoclaves combine both methods to sterilize. Gravity autoclaving is the most basic form and is suitable for sterilizing the most common laboratory media, including steel utensils, glassware, and bio-hazardous waste. Gravity autoclaves are also particularly advantageous when used in geographical areas of high humidity or higher altitudes. On the other hand, vacuum autoclaving is more suited in cases where air cannot be easily removed from sterilization media. This may include large or porous items such as animal cages and bedding sterilization.

Autoclaving cycles

As per India's bio waste management rules 2016, when operating a gravity flow autoclave, medical waste shall be subjected to a temperature of not less than 121° C and pressure of 15 pounds per square inch (psi) for an autoclave residence time of not less than 60 minutes; or 135° C , 31 psi for not less than 45 minutes; or 149° C , 52 psi for not less than 30 minutes. When operating a vacuum autoclave, medical waste shall be subjected to a minimum of one pre-vacuum pulse to purge the autoclave of all air. The waste shall be subjected to a temperature of not less than 121 ° C and pressure of 15 psi for an autoclave residence time of not less than 45 minutes; or 135 ° C, 31 psi for not less than 30 minutes.

Documentation and validation

SOP should be kept. Each autoclave shall have graphic or computer recording devices which will automatically and continuously monitor & record dates, time of day, load identification number and operating parameters throughout the entire length of the autoclave cycle. Otherwise manual log book should be kept.

Routinely indicator strips are used to ascertain proper autoclaving and it is necessary to stick over different locations of waste packs to ensure complete autoclaving.

a chemical indicator strip/tape that changes when a certain temperature is reached can be used to verify that a specific temperature has been achieved. It may be necessary to use more than one strip over the waste package at different location to ensure that the inner content of the package has been adequately autoclaved. Periodically biological indicator (*Bacillus stearothermophilus* in vials or spore strips, with at least 1×10^4 /ml) should be used to ascertain the quality of autoclaving. Annual inspection by the qualified technician should be carried out.

Safety practices

The hazards involved in the practice of autoclaving involve burns with steam, inadequate killing of infectious organisms, pressurizing and rupture of closed vessels inside autoclave or on opening of door. Hence certain precautions should be observed while autoclaving.

- i. Drain screen to be cleaned to allow steam circulation, door gasket to be checked for damage and should not be overloaded.
- ii. Autoclave should not be opened while in operation and should be opened when psi shows zero.
- iii. PPE such as heat resistant gloves, splash goggles to be worn.

ETP and its operational principle

The wastewater, otherwise known as effluent contains several pollutants, which can be removed with the help of an effluent treatment plant (ETP). The waste discharge quality standards differ according to the point of disposal. Some of the parameters in the water quality discharge standards include colour, 5-day biological oxygen demand (BOD₅), the chemical oxygen demand (COD), total organic carbon (TOC), total suspended solids (TSS), total dissolved solids (TDS), metals, phosphorus, total nitrogen, nitrate, ammonia, sulphur, sulphide, oil, grease and pH. Effluent can be treated in a number of different ways depending on the level of treatment required. These levels are known as preliminary, primary, secondary and tertiary (or advanced). The mechanisms for treatment can be divided into three broad categories: physical, chemical and biological, which all include a number of different processes. Common physical unit operations include among other processes screening, flow equalisation, sedimentation, clarification and aeration. Chemical unit processes include pH control, coagulation, chemical precipitation and oxidation. Biological unit processes involve microbial growth (e.g. activated sludge) and attached microbial growth (e.g. fixed film). Depending on the requirement, these processes are combined.

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Practical

Waste Management (Solid, liquid and Gaseous)

Jagadish Hiremath

Introduction:

Waste management is an important component of both biosafety and biosecurity practice in biosafety laboratory. Biohazard waste generated are mainly three types viz., solid, liquid and gaseous. It is requirement for biosafety laboratory level 2 and above to decontaminate solid waste by autoclaving where as liquid waste after primary decontamination procedure in lab is left to be treated in effluent treatment plant. All the contaminated air is filtered through HEPA filters before it is left to environment.

Objectives:

1. To demonstrate handling of **solid** waste in BSL2 laboratory
2. To demonstrate handling of **liquid** waste in BSL2 laboratory
3. To demonstrate handling of **gaseous** waste in BSL2 laboratory

Activity:

1. Visit to BSL2 to demonstrate solid waste management
2. Visit to Effluent Treatment Plant to demonstrate liquid waste handling
3. Demonstration of role of BSC and HVAC in filtering the air circulating in laboratory

12. Biosafety documentation, SOP and Manual

Divakar Hemadri

ICAR - National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI)

In a biological laboratory, biological agents such as microorganisms, their components or their derivatives are collected handled and/or stored. A biological laboratory can be clinical laboratory, diagnostic facility, regional and/national reference centre, public health laboratory, research centre (academic, pharmaceutical, environmental, etc.) and a production facility (manufacturers of vaccines, pharmaceuticals, large scale GMOs, etc) for human, veterinary and agricultural purposes. Handling of biological agents or their components always pose some amount of risk not only to their handlers but also to those in the vicinity and the environment. Therefore, any such laboratory dealing with these agents/materials need to protect protects their workers, animal populations, and the environment from exposure or spread of pathogens. In this way, the laboratory biosafety describes the principles and practices for the prevention of unintentional release of or accidental exposure to biological agents and their derivatives. Another common terminology that is used commonly with biosafety is laboratory biosecurity which describes the physical control of biological agents and toxins within laboratories, in order to prevent their loss, theft, misuse, unauthorised access or intentional unauthorised release.

Biological laboratories in the world ensure the protection of workers, animal populations, and the environment by means of strict imposition of certain guiding principles and procedures, which are well documented in the form of manuals, standards and standard operating protocols (SOP). Generally, these laboratories, formulate these policies, manuals and SOPs, based on the general policies and guidelines developed at the national level. In India, the biosafety and biosecurity policies are developed by Department of Biotechnology, Government of India. The institute level biosafety policies and procedures are put in place following the recommendations of institutional biosafety committee (IBSC) that is headed by the head of the institute.

In general, biological laboratories identify, implement and maintain suitable methodologies for biological risk assessments, including timelines, personnel and that the associated reporting and approval mechanisms. This is accomplished through the development of a risk management policy appropriate to the nature and scale of the facility, activities, and associated biological risks managed through well documented administrative, operational, engineering, and personal protective equipment (PPE) controls. Thus a complete and functioning laboratory biological risk management system will help ensure that the laboratory is in compliance with applicable local, national, regional, and international standards and requirements for biosafety laboratory.

Biosafety Documentation: SOP and Policies

Jagadish Hiremath

Introduction:

The practices of biosafety and biosecurity norms in the biocontainment laboratory needs various documents to establish the best practices in place. In this regards there are number of documents maintained to monitor the activities like laboratory personnel training, visitor policies, movement of materials, use of safety equipment, service registers, calibrations, validations, clearances, test reports etc.,

Objectives:

1. To discuss the need for different biosafety documents
2. To show the various documents maintained in BSL2

Activity:

1. Visit to BSL2 lab to show and demonstrate the various biosafety documents maintained in BSL2 for different activities at different locations

13. Biosafety practices: Collection, Package and Transportation of Infectious Materials (National)

P. Krishnamoorthy

ICAR - National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI)

Sample

The term “sample” may either mean a specimen (animal, blood sample and others) or, used in the statistical sense, a sub-collection or sub-set of units. The aim in general is collection of samples representative of the study population. It is difficult to collect materials required for disease diagnosis from all the animals in a population. Hence the samples are collected to represent the population; usually samples are collected at random. Clinical samples mean samples collected from an ailing or affected animals with particular diseases. The infectious materials includes all the pathogenic organisms including bacteria, virus, fungi, parasites, etc., and may be present in the clinical samples suspected for various pathogens.

Collection of samples

Sample collection is done mainly for direct examination of the sample from the animals, isolation of microorganisms for definitive diagnosis and serological diagnosis which will help in the diagnosis of animal diseases.

Collection of specimens for culture differs from collection of specimens for routine analysis.

1. Avoiding contamination by the organisms on the skin is essential if misleading results and inappropriate therapy are to be avoided.
2. Although any small volume can be cultured, the probability of obtaining a “positive” culture increases in proportion to the size of the sample obtained. Sub-optimal samples can result in the inappropriate diagnosis of disease.
3. Collect specimens in sturdy, sterile, screw-cap, leak proof containers with lids that do not create an aerosol when opened.

Aerobic Culture Swab

The use of the aerobic culture swab is recommended for most body sites for which a swab of such size is a suitable method to collect the specimen.

1. Collect the specimen and replace the swab in the plastic container.
2. If the collection swab has an ampoule, crush it to ensure that the transport fluid comes into contact with the swab. If the collection swab has a moistened sponge, no action is needed.
3. Label with animal number, age, sex, breed, body site, type of sample and date and time of collection.
4. Refrigerate specimen for transport. Deliver to lab within 48 hours.

Anaerobic Culture Collection

1. The sample should be collected from the active site of infection and precautions should be taken to exclude surface contamination and the aeration of the sample. Whenever possible, specimens should be pus or fluid obtained by needle aspiration through intact skin or mucosa, which has been cleansed carefully with antiseptic.
2. In situations where material must be obtained from open foci of infection, sinus tracts or drainage tracts, it is best to aspirate purulent material with a syringe attached a sterile plastic catheter. The assembly can be passed deeply into the sinus tract or wound after the surface opening has been mechanically cleaned with a non-germicidal agent.
3. If irrigation is required to obtain an adequate specimen, lactated Ringer's or non-bacteriostatic normal saline (sterile) may be used. Broth should not be used.
4. Swabs may be used only as a last resort and they must be stored in an oxygen-free atmosphere. As much specimen as possible must be taken up on the swab so that the tip is saturated. A second swab is used for the Gram Stain.
5. Tissue suspected of containing anaerobes should be placed in appropriate anaerobic containers.

Collection of blood and serum

Before collection of blood, make the area of skin surface sterile by using alcohol swabs. Collect blood from animals using sterile vacutainer tubes if blood is used for isolation of microorganisms. The vacutainer needles are very useful in collecting blood with less pain to animals. The whole blood may be collected in the tube containing Di-potassium EDTA coated or heparin coated tubes. Collect 5 to 8 ml of blood per animal which can be used for various tests and diagnosis of diseases. For serum, collect the blood in tube coated with sodium silicate vacutainer tubes which favours clot formation and easy separation of serum. The serum should be separated by centrifugation at 2000 rpm for 15 to 20 minutes. The serum collected may be used for the serological investigations and it should be stored at -20°C until used. Blood can be used for isolation of various organisms and also to extract DNA to confirm by PCR. Serum can be used for detection of antibodies against different pathogens if present.

Filter paper technique

Cut the filter paper into strips of size 4 cm x 1cm and pin at the center by using three strips. It makes the six ends for collecting the blood sample. Put few drops of blood on the filter paper strips and allow it to dry. Then the filter paper can be sent by post to the concerned laboratory for diagnosis of animal diseases. The dried blood can be used for isolation of DNA and confirmation of disease suspected by PCR may be done. This technique is an easy one and involves less transportation and storage requirements.

Tissue collection

The tissues samples are usually collected from the dead animals for identifying the cause of the death. Tissue samples immediately after collection it should be fixed in proper fixative for histological examination. Usually, 10 per cent formalin is used and it is prepared by adding 10ml

of 40 per cent formaldehyde with 90ml of tap water to make 100ml of 10 per cent formalin. In carcasses of rare species, preservation of organs *in situ* for anatomical studies may be useful, with only small samples taken out for examination, causing as little disturbance as possible. If a necropsy is only done for detection of health problems, enough tissue for examinations including bacteriology should be taken, samples including abnormal areas and surrounding normal areas. It is recommended samples no thicker than 1 cm (for good fixation), but long and wide enough to represent different areas of a tissue and possible abnormalities to be collected. In small animals, entire organs instead of samples may be collected. The mechanical damaging of samples by compressing them with forceps may be avoided.

Collection of nasal swabs

The nasal swabs can be collected from the posterior nasal passages using a sterile swabs moistened with transport medium or saline or phosphate buffer saline. The swabs should be collected by using readily available sterile swabs in plastic tubes and after collection it can be placed immediately in the culture tubes containing culture media for bacterial isolation purposes.

Collection of faecal swabs

The faecal swabs are collected for bacterial isolation or for determination of parasites in the faeces. The moistened swabs are inserted into the rectum and rotated clockwise and anticlockwise, collect the faeces and immediately put in the culture tubes for isolation of microorganisms.

Collection of skin scrapings

The skin scrapings can be collected using sterile scalpel and forceps and collected in the paper. It is collected mainly for examination of external parasites.

Collection of cerebrospinal fluid

The lumbo-sacral joint between the last lumbar vertebrae and the anterior end of sacrum is preferred site for collection of cerebrospinal fluid. The skin surface is shaved, washed with detergent and disinfected with alcohol. The sample is collected using the sterile spinal needle or 18 gauge needles with aspiration by 10 ml syringes.

Storage of clinical samples

1. The samples are stored if the processing is delayed and usually done using refrigeration.
2. The blood samples should be processed immediately but can be stored for 24 to 48 hours in refrigeration at 4° C.
3. The serum can be stored at freezing conditions at 0° C or -20° C for long time storage without decomposition of the serum proteins.
4. The tissues should be stored at -40 to -80° C if it is to be stored for long period.
5. All the clinical samples should be chilled to refrigeration immediately after collection if the processing is delayed.

Packaging of infectious materials or clinical samples

The infectious materials or clinical samples need to be properly packed using triple layer packaging method. Any packaging for biological substances must include three components:

- A primary receptacle: the tube, vial or other container typically made of glass or rigid plastic (including the stopper, cap or other closure elements) that is in direct contact with the specimen.
- A secondary packaging (including cushioning and other materials) that fully encapsulates the primary receptacle.
- An outer packaging for transportation.

At each layer include sufficient absorbent materials so as to absorb the infectious material/ clinical samples in case of accidental spillage. The main purpose of the packaging is to avoid the contamination and spread of the infectious agents to the personnel handling during the transportation.

Components must meet specific requirements, including being capable of passing specific test procedures based on receptacle or packaging type. In addition, compliance with the regulations is based, in part, on overall performance; so there can be no substitutions of a component from one manufacturer with a similar but untested component from another manufacturer.

Procedures to be followed during transport of clinical samples

1. Do not transport material for culture in the needle and syringe. Needle transport is very unsafe because there is always the risk of a needle stick injury, and syringe transport poses a risk because specimen may be expelled during transport, creating a threat to personnel and environment. Transfer aspirated material to a tight container.
2. Place tissue samples, biopsy samples into an anaerobic transport device or a sterile tube or petri dish. Place all of this into a sealable plastic bag that generates an anaerobic atmosphere which will prevent contamination.
3. The sample containers should be properly labeled and identified. The labels like “Clinical Specimen, Fragile and Handle with care” may be written on the container or the parcel containing the clinical samples.
4. The sample container should have the details like nature of the clinical sample, animal number, place and date of collection which is important for easy identification and tracking of samples.
5. The samples should be sent with proper sample details and history of the case in a separate sheet which will help in the appropriate tests to be carried out for particular diseases.
6. Blood smears should be dispatched after fixing in methyl alcohol or suitable fixatives.
7. The serum samples should be transported on ice at 4° C to prevent decomposition of the serum proteins.
8. The tissues samples should be transported in transport media or directly on ice as early as possible to prevent autolysis of the tissues.
9. Milk samples should be dispatched as quickly as possible, if dispatch is delayed then add 1 part of 5 per cent boric acid to 10 parts of milk as a preservative.

10. For isolation of virus, the specimens collected should be stored in 50 per cent glycerol saline or phosphate buffer solution containing 5 per cent bovine serum albumin and antibiotics.

Sample collection from animals for diagnosis of various diseases

Bacterial diseases:

Name of the disease	Symptoms of the disease	Clinical specimens of choice for laboratory diagnosis
Anthrax	A zoonotic disease caused by <i>Bacillus anthracis</i> , is characterized by high fever, bloat, respiratory distress due to oedema of thorax and brisket region, muscular tremors, abdominal pain and sudden death followed by bloody discharges from natural orifices.	<ol style="list-style-type: none"> 1. Blood smears from ear vein, swelling and discharges from natural orifices, peripheral blood, heart blood, spleen and swollen lymph nodes for demonstration of bacilli. 2. Ear tip or a piece of muzzle in saline for isolation of anthrax bacilli.
Brucellosis	A zoonotic disease causing contagious abortion and infertility. It is caused by <i>Brucella abortus</i> in cattle, <i>B. melitensis</i> in sheep and goats, <i>B. suis</i> in pigs.	<ol style="list-style-type: none"> 1. Milk, for milk ring test and isolation. 2. Serum sample (paired serum sample) for serological tests. 3. Vaginal mucus, uterine fluid and blood on ice for isolation and PCR. 4. Semen samples and swabs from the male reproductive organs for isolation and PCR. 5. Stomach contents of aborted foetus, on ice for isolation or PCR.
Campylobacter infection	Contagious venereal disease of cattle characterized by abortion, infertility with repeat breeding caused by <i>Campylobacter foetus</i>	Vaginal mucus swabs and preputial washing in sterile swabs and stomach content of aborted fetus on ice for isolation of Campylobacterium.
Black Quarter (BQ)	BQ is a disease of sheep and cattle and caused by <i>Clostridium chauvoei</i> bacteria. A symptom is characteristic swellings which make a crackling sound under pressure.	<ol style="list-style-type: none"> 1. Impression smears from the affected muscle and exudates from the swelling for demonstration of Causative organisms. 2. Pieces of affected muscle and intestines on ice for isolation of <i>Cl. chauvoei</i>
Enterotoxaemia (ET)	An infectious disease of ruminants caused by <i>Clostridium perfringens</i> and characterized by abdominal pain, hemorrhagic enteritis and sudden death. Symptoms vary depending upon the type of toxin produced by the organism (types A, B, C, D, E, F etc.).	<ol style="list-style-type: none"> 1. Smears from contents of small intestine for demonstration of Gram positive rods with spores. 2. Contents and pieces of small intestine, blood on ice for isolation of Clostridium.

Name of the disease	Symptoms of the disease	Clinical specimens of choice for laboratory diagnosis
Haemorrhagic Septicaemia (HS)	Caused by <i>Pasteurella multocida</i> and the disease is characterized by high fever, localized oedema and respiratory symptoms.	<ol style="list-style-type: none"> 1. Smears from peripheral blood, fluid from swelling, impression smear from heart, lungs, liver, submaxillary swellings for demonstration of bipolar organism. 2. Blood in sterile container for isolation. 3. Swabs from exudates, heart blood and pieces of liver, spleen and kidney, lymph nodes on ice for isolation of <i>Pasteurella multocida</i>.
Leptospirosis	A zoonotic disease caused by the different species of the Genus <i>Leptospira</i> . The disease is seen as an acute or chronic or clinically inapparent condition and is characterized by sudden fever, muscle tremors, anorexia, haemoglobinuria, icterus and abortion.	<ol style="list-style-type: none"> 1. Blood and serum for dark field microscopic observation, isolation and PCR of leptospire. 2. Tissue from kidney, liver and spleen in 10% formalin for histopathology. 3. Milk or urine in vials (on ice) for isolation.
Listeriosis	Listeriosis or circling disease is a fatal infectious disease of man and animals caused by <i>Listeria monocytogenes</i> . The disease is characterized by encephalitis, abortion or septicemia.	<ol style="list-style-type: none"> 1. Blood, cerebrospinal fluid, medulla and portion of spinal cord, brain tissue, aborted foetus or placenta on ice for isolation of listeria. 2. All internal organs in 10% formalin for histopathology.
Johne's disease (paraTB)	A chronic, infectious, fatal gastrointestinal disease of ruminants caused by <i>Mycobacterium johnei</i> . The most cardinal symptom is continuous or intermittent diarrhea leading to progressive emaciation and death.	<ol style="list-style-type: none"> 1. Rectal pinch swab or smear for demonstration of Johne's bacilli. 2. Faecal samples, terminal portion of ileum with ileocaecal valve on ice for isolation of acid fast organisms.
Bovine Tuberculosis (TB)	A chronic contagious disease of man and animals caused by different species of <i>Mycobacterium</i> . The disease is characterized by a painful, dry, hacking cough, respiratory distress, abdominal pain, diarrhea, chronic bloat, emaciation, irregular oestrus cycle, abortion, sterility, formation of small nodules in mammary tissues, painful swellings of the joints, etc.,	<ol style="list-style-type: none"> 1. Sputum and nasal swabs and milk in and lymph glands or lung lesions in sterile container on ice for isolation. 2. Heat fixed impression smears from bronchial lymph glands for staining. 3. Affected tissue like lungs in 10% formalin for histopathology.

Name of the disease	Symptoms of the disease	Clinical specimens of choice for laboratory diagnosis
Glanders	A zoonotic disease usually seen in horses caused by <i>Burkholderia mallei</i> . The disease is characterized by nasal discharge, formation of small nodules on upper respiratory tract mucosa and along the lymphatic channels of the skin and presence of ulcers on the skin.	<ol style="list-style-type: none"> 1. Nasal discharge and pus from skin lesions on ice for isolation of bacteria. 2. Impression smears of pus for Grams staining. 3. Affected tissues in 10% formalin for histopathology.
Mastitis	Caused by different species of bacteria in cattle, buffalo, sheep, goats, pigs	Milk samples (mid-stream) before onset of treatment in sterile vials on ice.

Viral Diseases:

Disease	Symptoms of the disease	Clinical specimens of choice for laboratory diagnosis
Food and Mouth disease (FMD)	The disease is caused by <u>picornavirus</u> , the prototypic member of the genus <u>Aphthovirus</u> infecting cloven hoofed animals and is characterized by high fever that declines rapidly after two or three days, blisters inside the mouth that lead to excessive secretion of stringy or foamy saliva and to drooling and blisters on the feet that may rupture and cause lameness.	<ol style="list-style-type: none"> 1. Vesicular epithelium or oesopharyngeal fluid in 50% Phosphate Buffered Glycerine for isolation of virus. 2. Sera sample for diagnosis.
Rabies	It is caused by <u>Rhabdoviridae</u> family which infect all warm blooded animals and highly <u>zoonotic</u> . The symptoms include slight or partial <u>paralysis</u> , cerebral dysfunction, <u>anxiety</u> , <u>insomnia</u> , <u>confusion</u> , <u>agitation</u> , abnormal behavior, <u>paranoia</u> , terror, <u>hallucinations</u> , progressing to <u>delirium</u> and death.	<ol style="list-style-type: none"> 1. Head / whole carcass on ice for demonstration of viral antigen, viral inclusions and isolation of virus. 2. Brain on ice for demonstration of viral antigen, viral inclusions and isolation of virus. <p><i>Note:</i> It is not advisable to open the skull by persons not protected by vaccination</p>

Disease	Symptoms of the disease	Clinical specimens of choice for laboratory diagnosis
Blue-Tongue (BT)	It is caused by Orbivirus which infect sheep, goats, cattle and important signs are high fever, excessive salivation, swelling of the face and tongue and cyanosis of the tongue. Swelling of the lips and tongue gives the tongue its typical blue appearance.	<ol style="list-style-type: none"> 1. Collect the blood in heparin or EDTA when body temperature is at its peak. Spleen, lung and lymph nodes on ice for isolation of virus. 2. Paired sera in sterile vials on ice for serological investigation. 3. Spleen, lymph nodes, intestine in 10% formalin for histopathology.
Infectious Bovine Rhinotracheitis (IBR)	Caused by BHV-1 and involved in several diseases worldwide and in cattle it causes <u>rhinotracheitis</u> , <u>vaginitis</u> , <u>balanoposthitis</u> , <u>abortion</u> , <u>conjunctivitis</u> , and <u>enteritis</u> .	<ol style="list-style-type: none"> 1. Sera for detecting antibodies by serological tests. 2. Swabs from vaginal, conjunctival and nasal lesions and pieces of trachea and lungs in 50% Phosphate Buffered Glycerine on ice for virus isolation. 3. Pieces of trachea, liver, turbinate bone, lungs in 10% formalin for histopathology.
Sheep and goat pox and Orf	Caused by a Capripox and parapox viruses, respectively. Symptoms include papules and pustules on the lips and muzzle, and less commonly in the mouth of young lambs and on the eyelids, feet and teats of ewes. The lesions progress to thick crusts which may bleed.	<ol style="list-style-type: none"> 1. Collect the blood at the height of body temperature in heparin or EDTA, scab and pustular materials, spleen, lung and lymph nodes on ice for virus isolation. 2. Paired sera in sterile vials on ice for serology. 3. Spleen, lymph nodes, intestine in 10% formalin for histopathology.
Peste des petitis ruminants	Caused by Morbillivirus. Infects goats and sheep and most typical signs are rise in body temperature, diarrhoea, ulceration of the buccal mucosae, especially on the inner face of the lips and neighboring gum, serous nasal exudates and conjunctivitis.	<ol style="list-style-type: none"> 1. Citrated blood, eye, mouth and rectal swabs and pieces of spleen, lymph nodes, intestine in PBS on ice for isolation of virus. 2. Sera samples for serological tests. 3. Lungs, liver, spleen, tonsil in 10% formalin for histopathology.
Swine Fever (CSF)	Swine fever causes fever, skin lesions, convulsions particularly in young animals and death within 15 days.	<ol style="list-style-type: none"> 1. Heparinised blood at the height of temperature for isolation, pieces of spleen, mesenteric lymph glands, intestine especially ileocaecal region in 50% glycerol saline for isolation of virus. 2. Pieces of brain, lung, intestines, ileocaecal region and kidney for histopathology.

Parasitic diseases:

1.	Theileriosis	Blood smears, biopsy smears from swollen lymph nodes from early stage of disease fixed with methanol
2.	Babesiosis/ Anaplasmosis	Thin blood smears fixed in methanol.
3.	Surra/Trypanosomiasis	Wet film examination of blood by hanging drop, fixed blood smears, blood in anticoagulant on ice.
4.	Schistosomiasis	Nasal schistosomiasis –Nasal discharge and nasal granuloma in normal saline.
5.	Trichomoniasis	Vaginal or uterine discharges, preputial scraping/ washing.
6.	Gastro-Intestinal Parasitic Diseases	Dung sample and affected internal organs in 10% formalin.
7.	Ectoparasitic Infestations (Ringworm, Mange, Mites)	Deep skin scrapings in sterile vials.
8.	External Fungal Infections	Skin scrapings in sterile vials

Thus the proper and systematic collection, dispatch and storage of samples are necessary and given at most care for proper diagnosis of animal diseases. The effort made in diagnosis will go waste if proper or representative samples are not collected. Hence the collection of samples should be given more importance during the diagnosis of animal diseases.

References

1. OIE Terrestrial Manual, 2009 by World organization for animal health, Paris, France.
2. Sample collection procedure manual, diagnostic services of Manitoba Inc., USA.
3. PD_ADMAS/Tech. Bull/8/2011 on Collection and dispatch of clinical samples for laboratory diagnosis of animal diseases.

Collection, Packaging and Transportation of Infectious Materials

Jagadish Hiremath

Introduction:

Collection, packaging and transportation of infectious materials from field to laboratory is an important activity which is done for various purposes like diagnosis, research and storage. Use of personal protective equipment at all levels of sample collection is important for the safety of an individual. Appropriate packing with recommended packaging type and materials is must to ensure the safety of the samples and people involved in the process of transportation. Use of signages/symbols on the shipment is critical to alert people handling it at various levels. This practical demonstrates the collection, packaging and transportation of samples from suspected anthrax cases in the field.

Learning Objectives:

1. To demonstrate the use of PPE during sample collection
2. To demonstrate triple layer packing of samples and use of various signages on the packed samples

Activity:

1. **The types of PPE to be used while collecting samples from anthrax suspected samples in field condition**

Before specimen collection

- Put on the chosen PPE in **clean zone**
- Ensure disinfectant, disposable bags, handwashing equipment's are ready
- Existing cuts/abrasions should be dressed before putting on PPE



After specimen collection

- rinse or wipe down gloved hands with 10% hypochlorite solution and discard outer gloves in to biohazard bag
- discard used PPE into disposal bags, separating autoclavable and non-autoclavable items
- inner gloves should be discarded last and sharps should be placed in a sharps container
- Wash hands.

2. **Sequence of triple layer packing of samples (Fig. 1)**

1. Collect the blood using EDTA **vacutainer**
2. Wrap the sample tube with **absorbable** cotton/tissue paper
3. The wrapped sample tube is inserted in to **Biohazard bag**
4. The biohazard bag with sample tube is placed in to sample box and packed for shipment with **biohazard sign**, detailed 'from' and 'to' address, instruction to handle carefully etc.,

Fig. 1



14. Biosafety practices: Collection & Transportation of Infectious Materials - International

Manjunatha Reddy, G.B

ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI)

Laboratory diagnostic results outcome depends mainly on type of sample collected, preservation technique and transportation along with finding suitable laboratory for performing the intended test or assay. The transport of biological materials, including infectious substances, is a subject of regulations governed at national or regional or international level. The international regulations for the transport of infectious substances by any mode of transport are based upon the Recommendations on the Transport of Dangerous Goods made by the Subcommittee of Experts on the Transport of Dangerous Goods (UN SCETDG), a subcommittee of the United Nations Economic and Social Council. The Recommendations are presented in the form of Model Regulations covering air, rail, road, sea and also include international mail. The World Health Organization (WHO) guidance document on “Transport of Infectious Substances” summarizing the different transport regulation is regularly updated. Apart from this, many countries have their own international organizations, international treaties and conventions such as the International Air Transport Association (IATA), the World Customs Organization (WCO), the Convention on International Trade in Endangered Species (CITES), and the Convention on Biodiversity (CBD), especially the Nagoya Protocol, provide additional guidance and regulations that should be considered in planning the transportation of biological materials. In the interest of animal and human health, biological materials collected from animals must be transported safely, efficiently and legally from the place where they are collected to the place where they are analyzed, studied or used.

The responsibilities

1. All the personnel involved in the packaging, labelling and shipping of biological materials must be appropriately trained, certified, competent and knowledgeable of the relevant national, regional and international regulations.
2. Biological materials should be transported to ensure a rapid and reliable system for delivery to the recipient using individuals such as professional logistics service providers that are trained and competent in the shipping and transportation process.
3. The efficient transport and transfer of biological materials requires co-ordination between the sender (shipper, consignor), the logistic providers, the carrier and the recipient (consignee) to ensure safe transport and arrival on time and in proper condition.
4. The sender (shipper, consignor) is responsible for providing the applicable documentation (e.g. certifications, permits) required by the national authorities of the countries of export, transshipment and import as well as ensuring that the shipment also complies with all other applicable regulations.
5. Procedures for incidents such as spills or theft of materials during transportation and any other realistic and foreseeable emergencies should be part of a risk management system in order to respond adequately to emergencies.

Maximum information is adopted from and for more details please refer chapter 1.1.3 on Transportation of biological materials in the OIE terrestrial manual 2018.

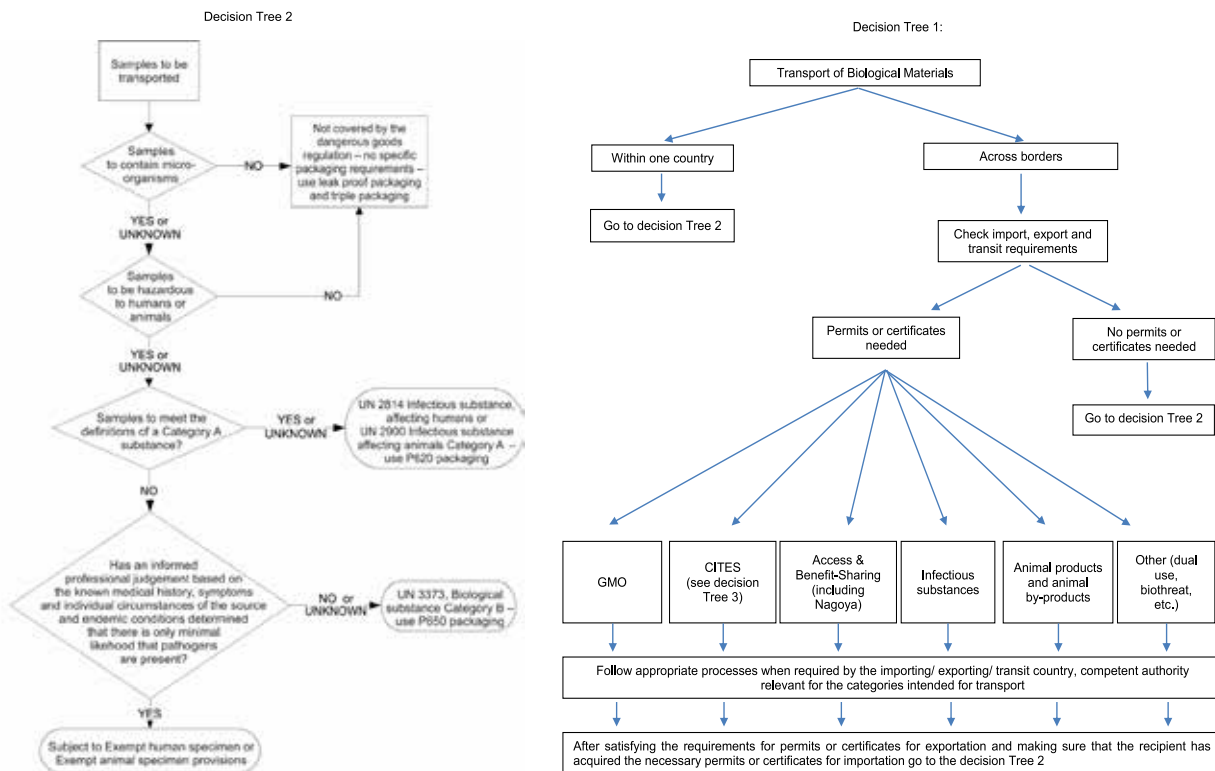
The transportation chain involves many more stakeholders with specific roles and responsibilities. These are explained in more details in the framework of aviation security in a joint ICAO (International Civil Aviation Organization) and WCO (World Customs Organization) brochure that can be accessed using the following link:

http://www.wcoomd.org/en/topics/facilitation/instrument-andtools/tools/~/_/media/4B167884A3064E78BCF5D29E29F4E57E.ashx.

In addition, Material Transfer Agreements (MTA) should be considered because they

1. Protect the interests of all involved parties in relation to;
 - a. Intellectual property
 - b. Potential alternative uses
 - c. Commercial aspects
 - d. Liability to third parties
 - e. Potential further transfers/uses
2. Help to avoid misunderstandings around the use of materials
3. Clarify ownership of property

Decision tree for the transport requirements of biological materials



Template of material transfer agreement which has to be amended depending on respective national or international requirements.

MATERIAL TRANSFER AGREEMENT

BETWEEN

PROVIDER

Organisation:
Address:
Country:

PROVIDER SCIENTIST

Title and name:
Organisation:
Address:

AND

RECIPIENT

Organisation:
Address:
Country:

RECIPIENT SCIENTIST

Title and name:
Organisation:
Address:

ORIGINAL MATERIAL

Description of the material being transferred

SHIPPING ADDRESS

Title and name:
Address:

15. Biosecurity: Physical, Personal, Pathogen and data security

Jagadish Hiremath

ICAR-National Institute of Veterinary Epidemiology and Disease Informatics

The laboratory biosecurity describes the protection, control and accountability for valuable biological materials (VBM) within the laboratory conditions with aim of preventing their unauthorized access, loss, theft, misuse, diversion or intentional release. The valuable biological materials are those which require strict access control, accountability and measure to monitor their overall use in the laboratory. These may include pathogens, toxins, vaccine strains and non-pathogenic organisms of economic or historical value. The VBM that received most attention, and indeed require protection in the context of laboratory biosecurity, are pathogens and toxins. The pathogen/toxin and procedures involved should be subjected microbiological risk assessment to identify the type and nature of pathogen, safety levels, biosafety practices and biosecurity measures to handling the pathogen. To achieve the recommended biosecurity measures it is important to adopt physical security, administrative, regulatory and procedures.

The aim of the physical security is to secure the facility against any natural odds and allow the access of authorized personnel/materials while restricting the unauthorized access of personals and materials. The physical security comprises facility location, perimeter security, the access control to facility, policies and procedures, intrusion detection and notifications at various places outside and inside of the laboratories. The physical security may focus on one more aspect depend upon the nature of facility.

Facility location: The location of laboratory facility is an important aspect of physical security. Laboratory building may be standalone or inside the main building, it may be in urban or rural settings, it may be in an area prone to natural calamities etc., All these factors has to be considered while identifying the laboratory location as well as managing the laboratory. The type of location determines the nature of physical security required.

Personal Security: The personnel working in biocontainment facility are primarily responsible either directly or indirectly in establishing and maintaining the biosecurity in classified laboratories. Hence, utmost care should be taken to select, train and monitor the laboratory personnel.

Pathogen security: The pathogen security comprises of many components like sample storage, inventory control, transfer control, transport control, pathogen inactivation and disposal. Sample storage in the laboratory can be secured with simple lock and key to be made available to authorized persons only. High end robotic technologies are also used to secure VBMs but they are costly for medium and small-scale laboratories. Inventory control is an important means to track the handling of samples into and out of storage places. Periodic verification of inventory is necessary to check for violations or unauthorized access to samples. Transfer and transportation of samples within and outside country should follow the national and international policies. Inactivation of infectious agents for disposal has to be done as per the recommended standard procedure. Ensure the inactivation process is complete by periodic validation of decontaminating equipment. The data recorded either manually in lab books or stored in computer should be secured and well protected.

References:

- ❖ ASM culture of responsibility workshop trainer's manual, American Society for Microbiology
- ❖ www.cdc.gov.gov/biosafety/biosecuritytraining/page53.html
- ❖ SafetyServices.ucdavis.edu/ps/bis/definitions

Practical

Laboratory Biosecurity Program

Jagadish Hiremath

Introduction:

The laboratory biosecurity describes the protection, control and accountability for valuable biological materials (VBM) within the laboratory conditions with aim of preventing their unauthorized access, loss, theft, misuse, diversion or intentional release. There are various systems in place to identify, monitor and prevent such activities.

Objectives:

1. To demonstrate the various tools, system, documents present in BSL2 that makes laboratory biosecurity program
2. To discuss the participants experience of laboratory biosecurity program in their country

Activity:

1. Visit to BSL2 lab to demonstrate the laboratory biosecurity system in BSL2 at ICAR-NIVEDI
Demonstration of use of CCTV footages in solving biosecurity issue

16. Agent Specific Biosafety and Biosecurity Practices: *Bacillus anthracis*

Jagadish Hiremath & Satish B Shivachandra

ICAR-National Institute of Veterinary Epidemiology and Disease Informatics

Bacillus anthracis is a naturally-occurring, rod-shaped Gram-positive, sporulating bacterium. Anthrax disease is endemic in animal populations in many of the SAARC member countries both in domestic animals as well as wild (e.g., cattle, sheep, antelope, elephants etc.). Anthrax disease can also occur in humans when exposed to infected animals or infected animal tissue (cutaneous or gastrointestinal anthrax) or when exposed to aerosolized anthrax spores (inhalation anthrax).

The *B anthracis* organism may be present in different clinical samples collected from human and animal cases. In human, the clinical samples viz., blood, skin lesion exudates, cerebrospinal fluid, pleural fluid, sputum, and rarely, in urine and feces the organism may be present. In animals the blood, tissue, bone marrow are rich in bacteria.

PPE: Personal protective equipment's (PPE) need to be worn by the veterinarian/laboratory technicians as well as animal handler's during disease outbreak investigation and handling of clinical materials. The recommended PPE in laboratory include lab coat, gloves, respirator (especially when soil samples are handled), coveralls, head cap and goggles are essential.

Biosafety and Biocontainment: The primary hazards to laboratory personnel are: direct and indirect contact of broken skin with cultures and contaminated laboratory surfaces, accidental parenteral inoculation and, rarely, exposure to infectious aerosols. Efforts should be made to avoid production of aerosols by working within the BSC. In addition, all centrifugation should be done using aerosol-tight rotors that are opened within the BSC after each run.

BSL-2 practices, containment equipment, and facilities are recommended for activities using clinical materials and diagnostic quantities of anthrax bacterial cultures. ABSL-2 practices, containment equipment and facilities are recommended for studies utilizing experimentally infected laboratory rodents. BSL-3 practices, containment equipment, and facilities are recommended for work involving production quantities or high concentrations of cultures, screening environmental samples (especially powders) from anthrax-contaminated locations, and for activities with a high potential for aerosol production. Workers who frequently centrifuge *B. anthracis* suspensions should use autoclavable aerosol-tight rotors. In addition, regular routine swabbing specimens for culture should be routinely obtained inside the rotor and rotor lid and, if contaminated, rotors should be autoclaved before re-use.

All the solid waste generated should be decontaminated using autoclave whereas the liquid waste should be treated with 1-2% bleach before it is disposed.

17. Agent Specific Biosafety and Biosecurity Practices: *Brucella spp.*

Rajeswari Shome

ICAR -National Institute of Veterinary Epidemiology and Disease Informatics (ICAR- NIVEDI),

Introduction to brucellosis

Brucellosis is one of the major bacterial zoonoses, affecting domestic animals and humans in many developing countries (McDermott et al., 2013) and WHO as recognized as one of the seven neglected, under-detected and under-reported zoonosis (World Health Organization, 2011). As per OIE, brucellosis has been classified under multiple species diseases, infections and infestations (OIE, 2016). The economic impact of brucellosis is due to productive and reproductive failures in livestock, loss of man days and morbidity in humans and countries economy impact is due to international obstacle to trade and export of animals and their products (Mcdermott and Arimi, 2002). Brucellosis has been eradicated from several countries in Northern and Central Europe, Canada, Japan, Australia and New Zealand (OIE, 2016). Although continuous progress has been made in brucellosis control in many parts of the world still it remains a major public health hazard of great economic importance causing an ever-increasing concern in many countries including India.

Why *Brucella* species are listed in biological select agent and toxins (BSAT)

Biological Weapons Anti-Terrorism Act of 1989 and the Antiterrorism and Effective Death Penalty Act of 1996, the United States Department of Health and Human Services (DHHS) developed a list of 31 infectious biological agents and 12 toxins that could pose a threat to public health. Criteria for inclusion on the biological select agent and toxins (BSAT) are effect of exposure on human health; the degree of contagiousness of the agent or toxin, mode of transmission, availability and effectiveness of therapeutics and vaccines; and any other criteria. Three species of *Brucella*, *B. abortus*, *B. melitensis*, and *B. suis* were included in the initial list of BSAT in 1996 due to their zoonotic capabilities.

Select agents and toxins are a subset of biological agents and toxins that may pose a severe threat to public health. *Brucella* species are easily aerosolized and have a low infectious dose, cited at levels between 10 and 100 microorganisms. These organisms also have a prolonged incubation period with the potential to induce a broad range of clinical manifestations, and therefore generate challenges for prompt diagnosis. The above factors have contributed to a select agent designation for *B. suis*, *B. melitensis*, and *B. abortus*.

Brucella as biological weapon/s

Some *Brucella spp.* (*B. melitensis*, *B. suis*, and *B. abortus*) have traditionally been considered to be potential biological weapons due to their ability to be transmitted by aerosol, cause chronic, debilitating zoonotic disease, and induce vague clinical symptoms that prevent rapid diagnosis. However, they also have zoonotic traits that make them less attractive as bioweapons including low mortality, long incubation periods, relatively mild clinical disease symptoms (“flu-like”), and being clinically responsive to treatment with a number of antibiotics including rifampicin or doxycycline. The incubation period is particularly prolonged for brucellosis, most cases

ranging from 1 to 4 weeks but in some instances extending to as long as 6 months. It has been suggested that the emergence of new, more virulent potential biological weapons make inclusion of *Brucella* among agents of bioterrorism to be mainly of historical significance.

Occupational risks of *Brucella*

The groups in which the occupational risk of infection is greatest include those whose work brings them in direct contact with infected animals or their products. These include farmers, stockmen, shepherds, goatherds, abattoir workers, butchers, dairymen, artificial inseminators, veterinarians and those involved in the processing of viscera, hides, wool and skins. Persons involved in the maintenance of buildings or equipment used for these purposes may also be at risk. An additional important category includes laboratory workers who may be exposed to contaminated specimens and to *Brucella* cultures, either during the course of diagnostic procedures or vaccine production, for example. The production and use of live vaccines also carries some risk.

Clinical exposure to *Brucella* Spp.

- ✓ Universal precautions and personal protective equipment (PPE) are essential when working with body fluids or tissues from a brucellosis infected animals and humans. When standard precautions are followed, most clinical procedures are considered to be low-risk activities.
- ✓ Higher-risk activities may include handling of tissues with potentially high concentrations of *Brucella* organisms (e.g., placental tissues), direct contact with infected blood and body fluids through breaks in the skin, or mucosal exposure to aerosolized *Brucella* organisms after an aerosol-generating procedure.
- ✓ Veterinarians and animal health care workers have a higher risk of contracting brucellosis because of close direct contact with infected animals and risk of exposure is greatest when veterinarians handle aborting animals or those undergoing parturition, though high-risk activities may also include specimen drawn during clinical examination, surgical procedures, or disinfection and cleaning of contaminated environments.
- ✓ Inhalation of aerosolized *Brucella* organisms and contamination of the conjunctiva or broken skin are common routes of exposure during the aforementioned high-risk procedures.

Laboratory exposure risks of *Brucella*

- Unknown or unidentified samples that arrive for analysis which poses greatest risk.
- Work performed on a *Brucella* isolate on an open bench, not under BSL- γ conditions.
- Certain characteristics of the bacterium, such as its low infectious dose and ease of aerosolization also contribute to the risk of infection by the organism in a laboratory setting.
- Sniffing bacteriological cultures, direct contact with cut or abraded skin, mouth pipetting, inoculations, sprays into eyes, nose, and mouth
- lack of experience working with the organism,

- Aerosol generating procedures include centrifuging without sealed carriers, vortexing, sonicating, accidents resulting in spillage or splashes (e.g., breakage of tube containing specimen).
- Other manipulations include automated pipetting of a suspension containing the organism, grinding the specimen, blending the specimen shaking the specimen and other procedures for suspension in liquid to produce standard concentration for identification (i.e., inclusion of steps that could be considered major aerosol generating activities)

Brucella vaccines exposure

- Accidental exposures to live, attenuated vaccine strains of *Brucella* spp. in veterinarians have been reported via needle stick injury, as well as through spray exposure to the conjunctiva and open wounds. Personnel administering RB51, S19, and Rev-1 vaccinations should wear proper PPE, including gloves and eye protection. Proper animal restraint should be used to minimize needle sticks or conjunctival splashes. The *B. abortus* RB51 developed to be less pathogenic and abortifacient than the S19 strain in animals, it does retain pathogenicity for humans. Local adverse events have been reported less than 24 hours after exposure, and systemic reactions may begin 1 to 15 days subsequent to exposure.
- *B. abortus* S19 Vaccine and *B. melitensis* Rev-1 Vaccine have been known to cause systemic disease in humans. Serological monitoring is available for S19 and Rev-1 exposures. Quantitative serological monitoring should be emphasized to detect a *B. abortus* S19 infection among veterinary workers, as patients may present with mild clinical symptoms or as asymptomatic.

Pet exposure due to *B. canis*

While dogs can become infected with various *Brucella* spp., they serve as the primary host for *B. canis* and it is thought to be less virulent than other strains of *Brucella* species and few human cases have been documented, though this may be a result of difficulty in diagnosis and underreporting.

Foodborne exposure

- ❖ Approximately 70 to 75% of U.S. brucellosis cases reported annually to CDC are due to *B. melitensis* and *B. abortus*, after individuals consuming unpasteurized dairy products from countries where brucellosis remains endemic. Areas currently listed as high-risk include: the Mediterranean Basin (Portugal, Spain, Southern France, Italy, Greece, Turkey, and North Africa), Mexico, South and Central America, Eastern Europe, Asia, Africa, the Caribbean, and the Middle East. Prevention measures should focus on educating immigrants and international travelers about the risks of consuming unpasteurized dairy products from these regions.
- ❖ Feral swine hunters who consume raw or undercooked pork are also at risk for food-borne exposure to brucellosis (via *B. suis*).

Biosafety and biosecurity practices to be followed at farms

- Farm workers, and animal attendants in particular, should wear adequate protective clothing when contact with infected animals is probable or if the environment is likely to have been contaminated by excreta, abortions or parturition products from animals with brucellosis. This is particularly important when dealing with animals that are aborting or giving birth, when the shedding of *Brucella* organisms will reach maximum levels.
- Aborted fetuses, placentae and contaminated litter should be collected in leak-proof containers and disposed of preferably by incineration. Deep burial in freshly slaked lime at sites away from water courses is an acceptable alternative. Any area in which an abortion or infected parturition has occurred should be washed down with an approved disinfectant (hypochlorite, iodophor or phenolic disinfectant at recommended working strength).
- Farm implements used for handling contaminated material should be disinfected after use by immersion in a suitable disinfectant (iodophor, phenolic soap or dilute caustic soda).
- Dung should be cleared daily and stored in a secluded area until rendered safe by natural decay (this will probably require about one year) or else burnt or soaked in disinfectant before disposal. Liquid manure can remain infected for long periods, especially at low temperatures. Destruction of *Brucella* organisms can be hastened by addition of calcium cyanamide or xylene but the material should still be stored for at least six months.
- Vehicles entering or leaving infected premises should pass through shallow troughs of disinfectant, or over straw or plastic foam soaked in an approved disinfectant.
- All persons carrying out high-risk procedures, which includes contact with animals suffering from or suspected of having brucellosis, should wear adequate protective clothing. This includes an overall or coat, rubber or plastic apron, rubber gloves and boots and eye protection (face shield, goggles or respirator).
- The risk of infection is greatest when dealing with aborting animals or those undergoing parturition but hazardous activities also include contacts with infected animals in other circumstances like shearing, dipping, clinical examination, vaccination and treatment, and the disinfection and cleaning of contaminated premises. The work clothes should be reserved for this purpose and retained on the premises.
- They should be disinfected after use either by heat treatment (boiling or steaming), by fumigation with formaldehyde or by soaking in a disinfectant solution of appropriate concentration (iodophor, phenolic soap, chloramine or hypochlorite).
- Particular attention should be given to the disinfection of footwear to ensure that infection is not transferred outside the premises or into the house or tent. Ideally, operatives should have access to full washing or showering facilities.
- As a minimum, the hands should be rinsed in a 1% chloramine solution (or other approved disinfectant), washed in soap and water and then treated with an emollient cream. Any superficial injuries such as cuts or scratches should be treated with an antiseptic, e.g. tincture of iodine, and covered with a bandage or self-adhesive dressing.
- Eye protection is particularly important as conjunctival contamination carries a high risk of infection. Should any infectious material enter the eye, it should be removed under clean or

aseptic conditions away from the working area. The eye should be thoroughly rinsed with running water and chloramphenicol or tetracycline eye drops or ointment applied. Respiratory contamination is also a high risk in heavily infected environments.

- Inhalation of dust or aerosols derived from dried excreta or tissues released at abortion, parturition or slaughter should be prevented by the use of suitable respirators. The filters, which must be capable of retaining bacteria, should be changed regularly and the equipment itself disinfected by chemical or moist heat treatment.
- Ideally, staff should be kept under medical surveillance with periodic serological examinations. It is strongly recommended that new staff provide a baseline blood sample before starting work. Any that develop clinical disease should be treated promptly. Young people under 18 years of age and pregnant women should be excluded from high risk occupations.

Safety measures in the laboratory for handling *Brucellae*

- ✓ A **biosafety level** is the level of the [biocontainment](#) precautions required to isolate dangerous [biological agents](#) in an enclosed facility. The levels of containment range from the lowest biosafety level 1 (BSL-1) to the highest at level 4 (BSL-4). The purpose of containment is to reduce or eliminate exposure to potentially hazardous agents. This is achieved by good laboratory technique and the use of appropriate safety equipment, including biological safety cabinets (BSC) and personal protective equipment (PPE). The three main elements of containment are laboratory practices and technique, safety equipment and facility design.
- ✓ *Brucellae* fall into WHO Risk Group 3, i.e. pathogens that pose a high risk to the worker involved, but only a low risk to the community. Brucellosis is in fact one of the most easily acquired laboratory infections. The degree of risk varies, not only with the virulence of the organism, *B. melitensis* and *B. suis* being the most dangerous for humans, but also with the numbers of bacteria in the material being handled.
- ✓ Blood samples and biopsy material for either serological or bacteriological diagnosis will rarely contain *Brucellae* in sufficient numbers to present a significant risk to personnel handling them but should still be handled with care at Biosafety level 2.
- ✓ However, after *Brucellae* have grown in culture, dangerous numbers of organisms are present and strict precautions are required. At the same time Biosafety level 3 facilities, practices and procedures are required. The same applies when handling birth products from animals.
- ✓ Clotted blood samples present little risk and milk samples only a slight risk. Membranes, fetal tissues and fluids may contain up to >10⁹ *Brucella* cells per gram, and similar numbers may be encountered in handling cultures grown in the laboratory.

Physical requirements for a laboratory handling of pathogenic *Brucellae*

- When handling cultures and other potentially highly infected materials such as membranes, fetal tissues and fluids, Biosafety level 3 is prudent. A separate room is required with only one entrance; a biohazard notice prohibiting the entry of unauthorised persons should be prominently displayed at the entrance.
- Ideally, the room should have a double-door entrance designed to provide an airlock. The ventilation should be arranged to maintain the pressure within the room at a slightly lower

level than its surroundings. Air from the room should be discharged to the exterior, well away from air intakes and opening windows, otherwise it must be sterilized by filtration or heat treatment. The walls should be impermeable and all windows sealed to allow disinfection and fumigation; it should be safeguarded against infestation with rodents or insects.

- The room must have a properly installed and tested Class II or III biological safety cabinet. The air exhaust from the cabinet should be so arranged as to avoid interference with the air balance in the room or within the cabinet when it is switched on. The room should have a sink, an autoclave and enough incubator space for all culture requirements. Hand washing facilities must be provided near the exit.

Biosafety and biosecurity practices to be followed at meat processing establishments

- ❖ If animals are known to be infected with *Brucella*, they should be slaughtered at abattoirs designated for that purpose, where the staff have been specially trained and equipped to deal with the risk. The slaughter men should wear full protective clothing including waterproof overalls or aprons, boots, respirators and goggles or face shields. Rubber gloves must be worn and chainmail guards should be used to protect against accidental cuts.
- ❖ Eating, drinking and smoking must be prohibited in the working area. Adequate facilities for disinfection of protective clothing, implements and for personal washing should be provided. If specially designated abattoirs are not available, the slaughter of infected animals should take place at the end of the working day, after slaughter of healthy animals has been completed.
- ❖ Tissues that are likely to be heavily infected, such as udder and genitalia, should be destroyed. Full cleaning and disinfection of the premises and the equipment must be performed at the end of each working day.
- ❖ Animal tissues and refuse for disposal should be retained in leak-proof containers such as plastic bags. It is recommended that such material be incinerated. Entry to the premises should be restricted to employees.
- ❖ Young persons under the age of 18 and pregnant women should not be allowed access. If possible, staff should be recruited from individuals known to have serological evidence of previous exposure to *Brucella*. The staff should be kept under medical surveillance and antibiotic therapy implemented for any who develop symptomatic brucellosis.
- ❖ All employees and especially women of childbearing age must be apprised of risks associated with *Brucella* infection. Information regarding conditions affecting immune status (e.g. pregnancy, immunosuppressive drugs, neoplasms, etc.) should be provided to workers. Personnel should be encouraged to self-identify to the organization's medical authority/health care provider so they may receive appropriate medical counselling and guidance. Education in safe and hygienic working practices and containment practices should be ongoing and is especially important for new staff.

Procedures to be followed during shipping of *Brucella* suspected samples

- ❖ Biosafety measures (protective clothing, mask, gloves and laminar workstation) should be followed while collecting and despatching specimens from animals suspected for brucellosis

- ❖ Do not transport material for culture in the needle syringe and glass vials. Needle transport is very unsafe because there is always the risk of a needle stick injury, and syringe transport poses a risk because specimen may be expelled during transport, creating a threat to personnel and environment. Transfer aspirated material to a tight container.
- ❖ Place tissue samples, biopsy samples into an anaerobic transport device or a sterile tube or petri dish. Place all of this into a sealable plastic bag that generates an anaerobic atmosphere which will prevent contamination.
- ❖ Blood smears should be dispatched after fixing in 70% alcohol, methyl alcohol or suitable fixatives.
- ❖ The serum samples should be transported on ice at 4° C to prevent decomposition of the serum proteins. Serum samples for serological testing should be sent in pairs, including an acute (≤ 7 days after symptom onset) and a convalescent-phase (14–35 days after symptom onset) specimen.
- ❖ For isolation of bacteria, specimens should be collected in *Brucella* selective broth (transport medium) or in 1-2 ml normal saline solution and despatched to reach laboratory on ice at the earliest.
- ❖ Organ samples should always be despatched in *Brucella* selective broth (transport medium) or directly on ice as quickly as possible to prevent autolysis.
- ❖ Milk samples should be dispatched as quickly as possible, if dispatch is delayed then add 1 part of 5 per cent boric acid to 10 parts of milk as a preservative.
- ❖ Cerebrospinal fluid, synovial fluid and fluids collected from the thoracic, abdominal or pericardial cavities should be submitted in sterile screw cap bottles without adding any preservative.
- ❖ All isolates and specimens for confirmation to other laboratories should be grown on solid media in tightly sealed containers (e.g. slants).
- ❖ PCR samples should be acute whole blood (≤ 4 days post symptom onset) collected in tubes containing any anticoagulant EXCEPT heparin. Samples for PCR should be frozen as soon as possible after collection and shipped on dry ice. Samples should be sent with sample submission forms.

Summary

Handling infected animals and diagnosis of brucellosis are bio-hazardous. Several biologic characteristics make *Brucellae* easily transmissible within the close confinement of the clinical microbiology laboratory and farms, including the facts that the infecting dose for humans and animals is low, and the organism may enter the body in many ways (e.g., through the respiratory mucosa, conjunctivae, gastrointestinal tract, or abraded skin). The agents like *Brucella* are having a greater potential to be easily disseminated by aerosolization, and can cause higher morbidity and mortality than typical organisms. Preventing exposures, especially to infectious aerosols, can be accomplished with the proper biological containments like Biosafety level 2 and Biosafety level 3. *Brucella* is the most commonly reported agent of laboratory acquired infection, biosafety level 2 practices is required for specimen processing and biosafety level 3 practices is must for all activities involving manipulation of *Brucella* cultures. All staff must be fully trained in the appropriate safety procedures and personal protective measures usage (disposable gloves, masks and protective clothing) during all cultural procedures.

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18. Agent Specific Biosafety and Biosecurity Practices: *Leptospira* spp.

V. Balamurugan

Principal Scientist, ICAR- NIVEDI, Bengaluru-560064, India

Leptospirosis – the synonym or cross-reference for this disease are Weil’s disease, Rat fever, Rice fever, Canicola fever, Haemorrhagic jaundice, Mud fever, Swineherd’s disease. It is a transmissible disease of animals and humans caused by infection with the spirochete *Leptospira*. It is one of the fastest re-emerging widespread and one of the leading neglected zoonosis in the world and is emerging as an important public health problem, which results in high morbidity and considerable mortality in areas of high prevalence. It affects a wide range of mammalian host especially humans and animals including cattle, buffalo, goat, sheep, horse, swine resulting in causing heavy economic losses to the farming community. This is mainly due to the great diversity of the *Leptospires*, and their ability to infect and survive in a wide range of animal hosts. *Leptospira* are found in a variety of mammals, including livestock, dogs, wildlife, and laboratory animals, and they, therefore, pose a greater risk to laboratory workers in animal facilities. Infection-related to occupational exposure usually is caused by accidental parenteral inoculation, direct or indirect contact with cultures or infected materials especially urine), and animal bites (Sewell, 1995) .

Causative agent: The causative agent for Leptospirosis is the spirochete *Leptospira*. *Leptospires* are spirochetes that may be saprophytes (free-living) found in freshwater or pathogens which may cause acute or chronic infection of humans and animals. *Leptospires* belong to the order *Spirochaetales*, family *Leptospiraceae*, genus *Leptospira*. In the genus *Leptospira*, there are 21 species distinguished into three clades of pathogens (9 species), intermediate (5 species) and non-pathogens (7 species) group. *Leptospires* are further based on its immune-reactivity pattern divided into serovars divergent Leptospiral lipopolysaccharide (LPS) structures; antigenically there are more than 250 serovars which are further clustered into 25 serogroups for convenience.

Epidemiology: Worldwide reach, endemic in tropical and subtropical regions, especially in developing countries, found in both urban and rural setting. Occupational hazard agriculture field workers, farmers, veterinarians, miners, animal husbandmen, laboratory workers handling infected rodents and other animals; outbreaks can occur among those exposed to water bodies such as lake/pond/river/pools contaminated by urine of animals.

Host range: *Leptospira* affects wide range of mammalian host humans, livestock, domestic and wildlife animals, etc., Humans are being incidental host, *Leptospira* has wide host range, the animals that commonly develop or spread leptospirosis are Rodents, Raccoons, Opossums, Cattle, Swine, Dogs, Horses, Buffaloes, Sheep, and Goats. Although they can be infected with and pathogenic serovar or species these animals may act as a reservoir or carrier for specific host adaptive serovars.

Health hazard to human: The clinical manifestation is often protean with Fever, headache, chills, severe malaise, vomiting, myalgia, and conjunctival suffusion; occasionally meningitis, rash and uveitis; sometimes jaundice, renal insufficiency, anemia and hemorrhage of the skin; clinical illness lasts 3 days to few weeks, often biphasic; may have asymptomatic infection; low case fatality rate. Major outbreaks are often reported in developing countries especially Southeast Asian countries. The infective dose is not clear. The mode of transmission includes a contact of

the skin or mucous membranes with contaminated water, soil or vegetation; direct contact with urine or tissues of infected animals; occasionally through ingestion of contaminated food or by inhalation of droplet aerosols of contaminated fluids. The incubation period is about 3 to 21 days. Direct transmission from person to person is rare due to the low rate of shedding in urine during an infection.

In the outside environment, the following control measures should be employed for control of leptospirosis. Avoid contact with contaminated water or soil environments or infected wildlife, especially rodents. Do not allow animals to drink from or enter contaminated bodies of water. Leptospirosis vaccines are available for pigs, cattle, and dogs and these vaccines help to prevent disease severity but may not completely prevent infection. For preventing illness, prevent contamination of living, working and recreational areas by the urine of infected animals. Control rodent populations in areas of human habitation. Do not allow animals to urinate in or near ponds or pools. Keep animals away from gardens, playgrounds, sandboxes, and other places children may play. Try to avoid walking in floodwater. If you have to, wear gumboots.

Risk group of the organism: The *Leptospira* has been placed under risk group 2, mandatory BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of known or potentially infective tissues, body fluids, and cultures. The housing and manipulation of infected animals should be performed at Animal Biosafety SL-2. Gloves should be worn to handle and necropsy infected animals and to handle infectious materials and cultures in the laboratory.

Physiochemical property of organism: Physically sensitive to moist heat/ autoclaving (121° C for at least 15 min) and is also killed by pasteurization. Once shed through urine, outside the host pathogenic *Leptospire*s persist for long-duration under even in low nutrient conditions, thus animal excreta should be appropriately sanitized and decontaminated before releasing to the environment. The organism is **inactivated by or** susceptible to 1% sodium hypochlorite, 70% ethanol, glutaraldehyde, formaldehyde, **detergents, and acid.** *Leptospira* is sensitive to penicillin, streptomycin, erythromycin, and tetracyclines *in vitro*.

Monitoring and surveillance of laboratory personal: The laboratory personal must be monitored for symptoms of illness and confirmed by serologically and molecular techniques or by isolation of *Leptospire*s from blood, CSF or urine. In case of accidental exposure to culture or infected tissue/fluids. Doxycycline treatment within 4 days of onset, the combination of Amoxicillin and erythromycin can be effective. Vaccines are not commercially available for use in human. Doxycycline administered orally during periods of high exposure may prevent disease. However, immunization of man has been carried out against occupational exposures to specific serovars in some countries, but not in southeast countries or SAARC countries.

Laboratory Hazards: Laboratory hazard of leptospirosis is a well-documented one. Earlier, reports showed about 10 death and 70 laboratory-acquired infections from experimentally infected rabbits (CDC and NIH, 2009; Miller and others, 1987). Another example includes a laboratory worker who accidentally sustained cut and exposed to culture (Sugunan and others, 2004). The ideal specimen for leptospirosis diagnosis is from the blood (first 7 days), or CSF (days 4-10) during acute illness or urine after 10 days, IF and ELISA can be used for detection of *Leptospire*s in clinical specimens for diagnosis.

The primary hazard in the laboratory are ingestion, accidental parenteral inoculation, and

direct contact of skin or mucous membranes particularly the conjunctiva, with cultures or infected tissues or body fluids (especially urine), are the primary laboratory hazards. Inhalation of aerosols of contaminated fluids is not well defined. Another hazard include direct and indirect contact with fluids and tissues of infected mammals during handling, care, or necropsy is a source of infection; in animals with chronic kidney infections, the agent is shed in urine in enormous numbers for long periods of time. Rarely, the infection may be transmitted by bites of infected animals. Standard Operation Procedures (SOPs) should be developed that minimize the potential exposure of responding personnel to potentially hazardous biological materials.

Containment requirements for *Leptospira*: Biosafety level 2 practices, containment and facilities for activities involving the manipulation of known or potentially infectious tissues, body fluids and the housing of infected animals (for all serovars). Protective clothing: Laboratory coat; gloves for the handling and necropsy of infected animals and when there is the likelihood of direct skin contact with infectious materials.

To maintain a safe work environment, it is important to assess the risks and dangers within the lab and reduce those risks by implementing Primary and Secondary Barriers or control measures. Primary Barriers include biological safety cabinets, fume hoods and other engineering devices used by laboratory workers/researchers while working with a biological hazard agent. Procedures are also a form of primary barriers because they implement SOP. Strict adherence to standard microbiological practices and techniques and Biosafety or operations manual that identifies the hazards, which help prevent direct contact between you and the agent. Ideally, if primary barriers are sufficient and effective, Secondary Barriers such as personal protective equipment (PPE) should not be contaminated. Secondary Barriers consist of personal protective equipment because these elements act as a physical barrier between your body and the materials you are working with. PPE is your last line of defense which means that any contact with the material is in large part due to failure of a Primary Barrier. PPE can be as basic as eye protection (safety glasses or goggles), gloves, and a lab coat or as complex as a BSL 2 “positive pressure suit” that completely isolates the employee from the laboratory environment.

Generally, this Biosafety level 2 is applicable to clinical, diagnostic, teaching and other laboratories in which work is done with a broad spectrum of indigenous moderate-risk agents. These agents are available within the community but are associated with causing human disease with varying levels of severity. Using good microbiological techniques, these agents can be used safely and often on an open benchtop. Biosafety cabinets should be available for BSL-2 work as it is prudent and good practice to work with BSL-2 agents in a certified Biosafety cabinet of the appropriate type and rating. BSL-2 is often appropriate for work regarding human-derived blood and tissues where human cell lines which may contain pathogenic organisms or materials might be present. Again, prudent practices suggest that additional levels of containment be used if specific hazards are unknown. In this laboratory, Primary Barriers: Gloves, Lab Coat (Decontamination required for reusable lab coats), Eye Protection, Face Protection and splash shields (*as necessary*). Secondary Barriers: Hand Washing sink available, Waste Decontamination Facilities, Biosafety Cabinet available for use. The recommended secondary barrier (s) will depend on the risk of transmission of specific agents. For example, the exposure risks for most laboratory work in biosafety level 1 and 2 facilities will result from contact with the agents or inadvertent contact exposures through contaminated work environments.

In case of spills - Allow aerosols to settle; wearing protective clothing, gently cover spill with

absorbent material (paper towel) and apply 1% sodium hypochlorite, starting at the perimeter and working towards the center; allow sufficient contact time (30 min) before clean up. All the material must be decontaminated before disposal; steam sterilization, incineration, chemical disinfection. Storage of clinical specimen and culture should be kept in sealed containers that are appropriately labeled. Similarly, while handling animal experimentation, precaution should be undertaken in Animal Housing Biosafety Level (ABSL) 2, and Experimental animals are to be housed separately (Animal Biosecurity), Inoculations should be performed in Biosafety Cabinet /Cage Changing Station. Experimentally infected animals must be properly euthanized and decontaminated before disposal.

General safety rules for Leptospira Laboratory

- In connection with the dangers of contamination, it is not permitted to smoke, eat or drink in laboratories, with the exception of specially allocated places.
- All procedures, which could involve chances of direct or indirect contact with the organism or clinical material, are very hazardous. The use of gloves and the wearing of lab coat are thus highly recommended.
- Hypodermic needles, scalpels, microscopic slides, and similar sharp objects should be deposited immediately after use in the allocated containers containing disinfectants.
- In order to reduce the chance of prick accident, hypodermic needles should never be replaced in the protective sleeves.
- All the test tubes and vials containing organism or patient material should be thoroughly closed and should be deposited immediately in the allocated containers containing disinfectants and should be autoclaved.
- When performing procedures whereby aerosol formation, splashing or powder formation can occur, it is mandatory to use protective devices such as safety goggles, face masks, gloves, covered shoes or and extractor hoods.

Safety Recommendations

- Keep the laboratory area and administration as far apart as possible. Make sure the workplace is orderly with an easily cleanable surface.
- Keep the benches and workplaces as empty as possible. Do not sit on worktops. Workplaces and floors must be kept tidy.
- Pipetting must never be done with the mouth. Use an automatic pipette or a pipette using suction-balloon.
- A clean, disinfected workplace is extremely important in the prevention of contamination. Make sure to wash your hands with soap and water, not only when leaving the workplace, but also preferably between procedures.
- “Working condition” is an established point of attention in the work-assessment, meetings of the department. Co-workers are expected to offer their own points of attention concerning working conditions at these meetings. Unsafe situations must be immediately reported to their supervisors.

- Proper disposal of clinical specimens, laboratory waste, and chemical waste is of fundamental importance, for both yourself and others.
- The transfer of culture from one laboratory to another must be done with appropriate precaution with detailed data sheet about the potential hazard of the *Leptospira* as per national or international regulation and guidelines
- In the event of an accident, where the staff is infected or believed to have a risk of infection, prophylactic medication is advisable. Precautions should be taken while handling fresh isolates, serum or blood from the suspected patients.
- Material transport policies within an institution and outside of the facility should be made available and
- Personnel adequately trained and familiar with regulatory and institutional procedures for proper containment, packaging, labeling, documentation, and transport of biological materials

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19. Agent Specific Biosafety and Biosecurity Practices: *Rabies*

Manjunatha Reddy, G.B

ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI)

Rabies is an acute, progressive and fatal zoonotic disease with serious public health and economic consequences in India. The disease is caused by rabies virus belongs to phylogroup-I of genus *Lyssavirus* and family *Rhabdoviridae*. While all warm-blooded animals appear susceptible to infection with RABV, relatively few species act as reservoir host, which means being capable of sustaining intraspecific transmission of host-adapted virus strains. Rabies is the 10th biggest cause of death due to infectious diseases worldwide. It is estimated that 2.5 billion people across 100 countries are at risk of contracting rabies. The annual death rate is around 50000-60000, with 99% occurring in tropical developing countries. Around 36% of the rabies related deaths occur in India every year with dog bites being responsible for 95-97% of these cases. The annual estimated number of dog bites in India is 17.4 million, leading to estimated 18 000–20 000 cases of human rabies per year. Across Asia the annual expenditure due to rabies, was estimated to be reaching to 563 million US Dollars. Non-reservoir hosts (spillover hosts) may be infected following transmission of virus from a reservoir host, but onward transmission in the spillover host population is rare, resulting only in short chains of transmission and extinction of the virus in the spillover population when it does occur. In the urban form, dogs play an important role as the reservoir and transmitter of the disease to humans and domestic animals, while jackals and foxes maintain the virus in sylvatic form. Worldwide, transmission from dogs accounts for more than 90% of human cases. In developed countries, bats, foxes, coyotes, raccoons, and skunks are major reservoirs. Rapid and accurate laboratory diagnosis of rabies in humans and other animals is essential for timely administration of post exposure prophylaxis. Within a few hours, a diagnostic laboratory can determine whether an animal is rabid or not and inform the responsible medical personnel.

Risk group: Rabies is an occupational risk (group 2) for personnel who closely work with pets (veterinarians and their technicians/assistants, pet groomers, animal catchers and their transporters, laboratory animal researchers and technicians, animal control officers etc.), livestock (farmers, farm workers, veterinarians, veterinary technicians/assistants, lab animal researchers, slaughterhouse workers, meat processors etc.) and wild animals (hunters, tourists, forestry personnel, wildlife biologists, veterinarians and animal caretakers in zoo etc.)

Biosafety level: Biosafety Level 2

Biosafety practices: The disease is mainly transmitted through the bite by rabies infected animal. The other potential non-bite modes of transmission should be considered while handling the rabies infected materials in the laboratory which include contamination of a pre-existing wound, contact of mucous membrane or respiratory tract with an infected materials and exposure to aerosolized rabies virus. The biosafety practices are personal protective equipment includes but is not limited to laboratory coats or gowns, disposable gloves, protective masks and safety glasses. Pre-exposure vaccination of all the people working with rabies virus research, vaccine production and quality control. The materials suspected or confirmed for rabies should be stored in sealed, leak-proof containers with appropriate labelling and locked. In case of any spills, allow aerosols to settle and, while wearing protective clothing, gently cover the spill with paper towels and apply appropriate disinfectant starting at the perimeter, working inwards towards the centre with sufficient contact

time before clean up. Decontaminate of all infectious and non-materials used during laboratory work before disposal. Special practices may be recommended based on risk assessment.

Biosecurity practices: The biosecurity practices at field level includes pre-exposure immunization of individuals at high risk for exposure as described in risk group section. The personal protective equipment like front coats or gowns, disposable double layered gloves, protective masks and safety glasses while handling or doing postmortem examination of rabies suspected or confirmed cases. The post exposure prophylaxis consists of a multimodal approach to decrease an individual's likelihood of developing clinical rabies after a possible exposure to the virus. Regimens depend on the vaccination status of the victim and involve a combination of wound cleansing, administration of the rabies vaccine, and administration of human rabies immune globulin. If used in a timely and accurate fashion, post-exposure prophylaxis is nearly 100% effective.

20. Agent Specific Biosafety and Biosecurity Practices: Transboundary Animal Pathogen- *African swine fever virus*

Jagadish Hiremath

ICAR-National Institute of Veterinary Epidemiology and Disease Informatics

About the Disease: African swine fever (ASF) is highly infectious and deadly disease (100% mortality) of pigs caused by African swine fever virus which is dsDNA virus of genus *Asfivirus* and family *Asfarviridae*. The disease is contagious and rapidly spreading transboundary disease which was reported first time in China during August 2018. The disease is spreading rapidly among South-East Asian countries with latest report of outbreak from Myanmar.

Clinical Signs and Epidemiology: High fever, weakness, diarrhea, respiratory distress, nasal and conjunctival discharge and abortion in pregnant Sow. The incubation period range from 5 to 21 days with average of 5 days. The susceptible species in India includes domestic and wild pigs. All the breed and age groups are equally susceptible for the disease.

Transmission: : Direct or Indirect (Tick- *Ornithodoros sp*) contact with infected animals, bite of infected ticks, contaminate meat, fomites (premises, vehicles, implements, clothes) are sources of infection. The infected animal sheds virus from body fluids like blood, tissues, secretions and excretions.

Biosafety practices: The disease is exotic to India with potential to cause transboundary transmission. The African Swine Fever virus is risk group 4 organism requiring BSL3 and above biocontainment facility to handle the virus. The ASF is not a threat to public health. The biosafety practices are to be followed as per the requirement of biosafety laboratory level 3.

Biosecurity practices: The countries that are free from ASF should place strict laboratory biosecurity practices to prevent unauthorized access, loss, theft, misuse, diversion or intentional release of the virus.

Biosecurity at field level: Strict biosecurity measures at the borders of infected country may help to prevent or slow the spread of ASF. The pig farmers, transporters, veterinarians should observe a strict biosecurity measures during incidence of suspected incidence of ASF. Preventing exposure of pigs to ASFV infected blood, fluids, carcasses is critical to prevent ASF transmission.

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21. Bio-security and Bio-safety practices in surveillance of emerging tick borne diseases

Mohammed Mudassar Chanda, V R Kundave, D K Suresh,
N Balakrishnan and Jagadish Hiremath

ICAR-National Institute of Veterinary Epidemiology and Disease Informatics

Ticks are obligate blood feeding arthropods of animals, birds and humans. All stages of the life cycle of both sexes viz., larvae, nymph and adults require blood meal. Ticks can transmit many diseases to animals and humans. Ixodid (hard) ticks are characterized by the presence of a scutum (dorsal shield) and the capitulum at the terminal end.

KFD (Kysanur Forest disease)

Kyasanur forest disease is a viral disease affecting humans. KFD virus belongs to family *Falvivirus*. Even though KFD was once prevalent in the forested areas of Shimoga district of Karnataka state, recently it has been reported from seven other districts as well as from neighbouring states. The disease is mainly transmitted by the bite of infected ticks of genera belonging to *Haemaphysalis*. People visiting forests in endemic areas, Veterinarians are the main at risk group for KFD. There are no reports of human to human transmission of KFD (Pattnaik 2006; Heymann 2004; Borio et al. 2002; Murhekar et al. 2015). The case fatality rate (CFR) of human KFD cases normally range between 2 and 10% (Brown et al. 2005; Acha & Szyfres (2003); Heymann 2004; Gould & Solomon 2008). KFDV is high risk category pathogen requiring Biosafety level-4 handling facilities. However, handling of infected ticks or rodent samples collected in inactivating agents does not require BSL4 containment facility (Guidance from Pan American Health Organisation and the World Health Organisation: “General procedures for inactivation of potentially infectious samples with Ebola virus and other highly pathogenic viral agents”). Many studies on KFD using tick samples have been conducted in BSL2 or BSL3 facilities for diagnostic purpose (Wang et al. 2009; Charrel et al. 2005; Dodd et al. 2011; Murhekar et al. 2015). However, processing of samples for virus isolation should be strictly be carried out in BSL-4 containment facility.

Potential biosecurity risks while undertaking studies on KFD virus

- a. *Field level:* In field environment the exposure to infected tick bites and direct exposure to infected rodents poses more risk of acquiring KFD infection.
- b. *Laboratory risk:* Routes of infection in laboratory can be through accidental inoculation, ingestion and inhalation of aerosols generated while culturing the virus (Burke & Monath 2001; Banerjee et al. 1979; Bronze & Greenfield 2005; Morse et al. 1962).

Risk mitigation strategies

Personal protection:

The personnel involved in collection of tick samples must be vaccinated against KFD virus and should use full PPE (Personal Protection Equipment). PPE will comprise full body cover, including robust footwear with oversocks worn over thick trousers, and thick vinyl gloves with cuffs sealed with tape to prevent tick access. Tick repellents must be applied extensively on clothing 30 minutes prior to field visit using Permethrin (0.5%) and DEET (up to 30%) based repellents proven to be effective against *Ixodes* ticks (Cisak et al. 2012). PPE will only be used in the field and PPE must be removed prior to entering any vehicles or buildings, and stored in sealed plastic boxes within vehicles.

Tick sampling

Ticks must be removed directly from cattle or rodents, or from heel flags using metal forceps and should not be handled directly.

Rodent sampling

Rodents must be restrained using wire handling cones and handled using thick leather gloves to prevent any contact. Blood samples should be collected from restrained rodents using sterile, disposable syringes and sharps disposed of immediately into a puncture resistant container and incinerated after each field session.

Storage and packing

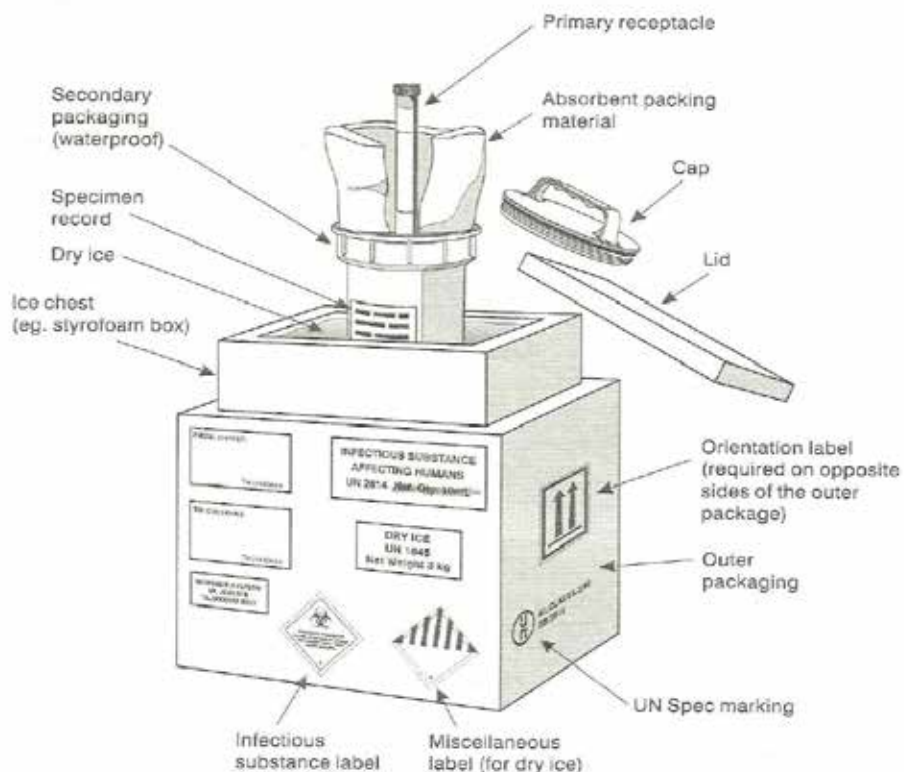
The tick and rodent samples should be immediately transferred to sure-lock sample containers containing combined Buffer AVL (Qiagen, Valencia, CA) and 80% ethanol. This has been shown to cause total viral inactivation in 100% of samples tested for viability of Ebola virus in clinical samples (Blow et al. 2004; Smither et al. 2015). Blood samples must be collected into sure-lock tubes containing buffer AVL and ethanol. Inhalation and contact with AVL can be harmful but the risk of this will be mitigated by the use of very small amounts in tubes, the opening of tubes only in well-ventilated field conditions, and also by the use of gloves and face mask (preferably N95).

Transportation of tick samples

The sample containers must be packed in triple layer packaging and transported to laboratories for further processing

Appendix 5: UN specification mark and labels

An example of the outerbox for Category A infectious Substance with dry ice.



Bio-safety protocols at the laboratory

Once the samples are collected in AVL buffer for complete virus inactivation, it can be handled in BSL2 + or BSL 3 laboratories. All the samples should be processed in Biosafety cabinets. Prior to processing, tick samples should be sealed in the transportation packaging for ten days at 4°C prior to allow AVL and ethanol to fully penetrate the ticks and inactivate the virus. The tick identification should be carried out in bio-safety cabinets using stereozoom microscope. The identified species will be stored and sealed in sure-lock tubes. RNA/DNA extraction must be similarly conducted inside bio-safety cabinets. Full laboratory PPE must be worn at all times: lab coat, N95 mask and gauntlet style vinyl gloves. After use, gloves will be removed aseptically. Personnel will wash their hands after handling infectious materials and before they leave the laboratory working areas. Any accidental spillage of blood, or serum during handling inside bio-safety cabinets must be decontaminated with Virkon (or a similar product) coupled with fumigation of the cabinet depending on the size of the spill.

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22. Agent Specific Biosafety and Biosecurity Practices: *Parasitic Diseases*

P. P. Sengupta and Siju Susan Jacob

ICAR- National Institute of Veterinary Epidemiology and Disease Informatics, Yelahanka,

Introduction: Parasitic diseases including protozoan and helminthic diseases are drawing increasing attention especially in developing countries because of their importance in immigrants, travellers and immune-compromised individuals. Persons working in research and clinical laboratories are at the risk of getting infected by parasites through accidental exposure that may not be recognised at the time of occurrence. Here we will discuss about major parasitic diseases that urge bio safety and biosecurity measures to properly manage like toxoplasmosis, cryptosporidiosis, cysticercosis and echinococcosis/hydatidosis.

1. Toxoplasmosis:

Toxoplasmosis is caused by *Toxoplasma gondii* which is an obligate intracellular protozoan parasite. Felines are the only definitive host and are the only animals that can pass the infective oocysts in their faeces. Warm blooded animals including human are intermediate hosts that can harbour tissue cysts in their bodies. Infection in human is initiated through the ingestion of oocysts containing sporozoites through contaminated food or water. Following ingestion, sporozoites invade the intestinal epithelium and differentiate to rapidly multiplying cyst stage (tachyzoites) followed by slowing multiplying cystic state (bradyzoites).

Pathogenicity: The organism is highly pathogenic in immune compromised individuals. The pathogenesis is due to proliferation of tachyzoites destroying host cells at a faster pace. If the infection affects optical tissue, it can cause improper vision, blindness, retinochoroiditis, microphthalmia, corneal opacity and cataract (Ocular toxoplasmosis). The parasite is having high affinity to placental tissue and in pregnant women rigorous multiplication of the parasite in the placental tissue can cause placental detachment thereby foetal death and other abnormalities including mental retardation, malformations etc.

Source of infection:

- Consumption of improperly cooked infected meats (pork, mutton, beef)
- Ingestion of food or water contaminated with oocysts
- Consumption of contaminated raw vegetables without proper washing
- Trans-placental transmission if the mother is having acute infection during pregnancy.
- Children can be infected via contaminated sandboxes or playgrounds

Risk group: Risk group 2

Containment requirements: Containment level 2 facilities, equipment and operational practices.

Susceptibility to disinfectants: Tachyzoites and tissue cysts are susceptible to detergents including 1% sodium hypochlorite and 70% ethanol. Oocysts are resistant to most of the disinfectants and 10% formalin is found to reduce the viability of oocysts. Decontaminate all wastes that contain or have come in contact with the organism before disposing by autoclave, chemical disinfection or incineration.

2. Cryptosporidiosis:

Cryptosporidiosis in human is caused by *Cryptosporidium parvum*, an intracellular protozoan parasite. It has a complex life cycle with sexual and asexual stages of the life cycle taking place within the same host. The parasite is having a wide host range. Preweaned calves are highly susceptible to infection and have been reported worldwide. Cryptosporidiosis in human is mainly reported in immune compromised individuals especially HIV patients.

Pathogenicity: Cryptosporidiosis is characterised by acute gastroenteritis with symptoms of diarrhoea, abdominal pain, cramps, vomiting, flatulence, anorexia, malaise and fatigue. Cryptosporidiosis is in the top list of five most common causes for infectious diarrhoea across the globe (Boatright and Greenfield 2005). In immune-competent individuals the illness is self limiting. The organism is classified as class B bioterrorism agent by the U. S. Department of Homeland Security.

Source of infection:

- Contaminated food or water- Water pipeline or water reservoirs contaminated with cattle faeces may act as source of infection
- Unwashed raw vegetables used in salads

Risk group: Risk group 2

Containment requirements: Containment level 2 facilities, equipment and operational practices.

Susceptibility to disinfectants: *C.parvum* is susceptible to high concentration of hydrogen peroxide, ethylene oxide and ozone. Decontaminate all wastes that contain or have come in contact with the organism before disposing by autoclave, chemical disinfection or incineration.

3. Cysticercosis:

The infection is caused by intermediate stage of the tapeworm *Taenia solium*. Human can act as both intermediate and definitive host for the parasite whereas pigs can acts as intermediate hosts. The adult parasite remains in the human intestine and eggs are passed through human faeces. Pigs can get the infecting by ingestion of human faeces while scavenging and the intermediate stage (*Cysticercus cellulosae*) develop in the muscles of pigs (measly pork). Human can get the infection either by ingestion of improperly cooked pork or by accidental ingestion of eggs (autoinfection). In case of autoinfection, human can act as intermediate host and the cyst will develop in the central nervous system (neurocysticercosis).

Pathogenicity: Infection with the tapeworm (taeniasis) in human is accompanied by diarrhoea, weight loss and discomfort. Neurocysticercosis caused by larval stages of the parasite is a serious concern with neurological manifestations including headaches, confusion, ataxia, seizures. Neurocysticercosis is recognised as the leading cause of the adult onset epilepsy.

Source of infection:

- Ingestion of raw unwashed vegetables contaminated with eggs
- Ingestion of undercooked pork infected with cysticerci

Risk group: Risk group 2

Containment requirements: Containment level 2 facilities, equipment and operational practices.

Susceptibility to disinfectants: Susceptible to 1% sodium hypochlorite and 2% gluteraldehyde. Cooking of pork above 60°C can inactivate the cysticerci. Decontaminate all wastes that contain or have come in contact with the organism before disposing by autoclave, chemical disinfection or incineration.

4. Echinococcosis:

Echinococcosis caused by *Echinococcus granulosus*, smallest tapeworm found in the intestine of dog. The disease is also known as hydatidosis. Man and herbivores generally acts as intermediate hosts. The intermediate stage (hydatid cyst) occurs most commonly in liver lungs, spleen, kidneys, heart, bone, and central nervous system.

Pathogenicity: Clinical manifestations of the disease are variable and depend on the location, size, and condition of the cysts. Symptoms of hepatic echinococcosis include hepatic enlargement, right epigastric pain, nausea, and vomiting. Release of hydatid fluid, due to spontaneous rupture of the cysts can result in allergic reactions ranging from mild to fatal anaphylaxis. Multiple secondary infections can occur due to dissemination of *E. granulosus* protoscolices.

Source of infection:

- Ingestion of food or water contaminated with *E. granulosus* eggs
- Through hands contaminated with egg-containing soil, sand or hairs of infected dogs
- dogs acquire infection through ingestion of cysts

Risk group: Risk group 2

Containment requirements: Containment level 2 facilities, equipment and operational practices.

Susceptibility to disinfectants: *Echinococcus* eggs are susceptible to 3.75 % of sodium hypochlorite (Krauss et al., 2003). Decontaminate all wastes that contain or have come in contact with the organism before disposing by autoclave, chemical disinfection or incineration.

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23. Biosafety considerations for handling Glanders

Dr. Praveen Malik

Animal Husbandry Commissioner (Offctg)

Director, CCS National Institute of Animal Health,

DAHD, Ministry of Fisheries, Animal Husbandry and Dairying, Govt of India

e-mail: malikphisar@hotmail.com

Glanders is a notifiable contagious and fatal disease in horses, donkeys and mules. It has been defined as an infection with *Burkholderia mallei*. It is considered a potentially lethal and dangerous zoonosis in people with a fatal outcome if not treated in a timely manner. Unprecedented emergence of glanders in several countries throughout the Middle East, Southern Asia, parts of Africa, and South America were reported in the past decade. Following the re-emergence of glanders in northern states of India during 2006, the disease has recently spread to several states of India affecting hundreds of equines. Based on the classification of microbes in four risk groups, *Burkholderia mallei* falls under risk group 3 and it requires BSL-3 level of containment.

Burkholderia mallei is Gram-negative bacillus, aerobic, non-motile, bipolar staining, non-sporulating and non-capsulated. It exists primarily in infected host and can withstand drying for 2-3 weeks in dry conditions. It can be killed by sunlight, high temp and various disinfectants. The organism affects solipeds, donkeys and mules mainly in acute form while horses in chronic form. Carnivores, humans, camels and goats are also susceptible while swine and cattle are considered resistant. The disease can be transmitted by Ingestion, which is considered the major route in animals, inhalation and direct contact. The transmission is enhanced by shared food and water facilities. Laboratory-acquired glanders has been reported in humans. The organism is a potential bioweapon, categorized in category B, due to the fact that very few organisms are required to cause disease, aerosols are easily produced and pulmonary form has high mortality. Diagnosis in animals is based on clinical signs and symptoms, pathological findings, mallein testing, laboratory testing including serology (CFT, ELISAs and western blot), lab animal inoculations and PCRs.

The disease appears to show three forms: nasal, pulmonary and cutaneous. In most outbreaks, these forms are not clearly distinct and may occur simultaneously in an animal. The disease can also occur in acute, chronic or latent form. Chronic and latent forms are more common in horses, whereas the acute form is more common in donkeys and mules. The latent state is particularly interesting as the agent, *B mallei*, is capable of entering a dormant state within the reservoir host (generally considered to be the horse). It appears that current diagnostic methods are unable to detect the organism during this latency. Therefore, detection of these inapparent carrier animals is a major challenge, and development of adequate diagnostic tests that can identify this asymptomatic state is essential for glanders control. The preparation of the protein fraction (mallein) in 1891 and the use of mallein in an intradermal diagnostic test opened the way for experimental diagnosis of glanders. The complement fixation test (CFT) for glanders diagnosis was then developed in 1909. Currently, the diagnosis of glanders in equids is based on either detection of the aetiological agent/ its genetic material or serology excluding the delayed hypersensitivity reaction (mallein test) and advanced molecular biology tools. CFT is among the most accurate and reliable serological tests for diagnostic use to date. Different ELISA formats including avidin-biotin Dot-ELISA, cELISA and indirect ELISAs have been developed for diagnosis of glanders. However, these assays use either crude or purified bacterial fractions as antigens thereby affecting the test specificity. Two indirect ELISAs based on recombinant proteins, namely *Burkholderia* intracellular motility protein A (rBimA), TssB and Hcp have shown a promising sensitivity and specificity. A cross-reactivity experiment with melioidosis serum from people and equids with disease and control serum showed that the recombinant antigens were highly specific to *B mallei* antibodies. An immunoblot assay based on partly purified lipopolysaccharide (LPS) containing the antigen of three

B mallei strains (Bogor, Zagreb and Mukteswar) has been developed. Recently, these ELISAs and the immunoblot assay have been evaluated using a significant number of positive sera, and have been internationally validated as per OIE guidelines.

The treatment of the disease in animals is not recommended in animals for obvious practical reasons, although the antibiotics are effective against the organism *in vitro*. The control is recommended by surveillance and test and elimination as per National Action Plan (2019) of the DAHD, Govt of India, in accordance with the 'Prevention and Control of Infectious and Contagious Diseases in Animals Act 2009'. Veterinarians, groomers, horsemen, butchers (where horses are also hatched for meat) and lab workers are major risk groups in humans.

In humans, the disease causes localized cutaneous lesions, pulmonary infection and septicemia and may take a chronic course. Generalized symptoms include fever, malaise, muscle aches and chest pain. Case-fatality rate may reach 95% in untreated patients. Since the human cases are not common, the treatment strategy in humans is based on limited experience and experimental data. Long term antibiotic treatment is recommended which may extend up to 12 months. Drug of choice is TMP-SMX, Imipenem, Cluvlanate, Azithromycin and Doxycyclin (combinations). No proven pre- or post-exposure prophylaxis or vaccine is available so far. Elimination of disease in animals and education and awareness among equine owners, handlers, veterinarians and laboratory workers form the basis for prevention.

All procedures with this organism must be conducted within Biological Safety Cabinet (BSC) either class I or class II. Under certain exceptional circumstances, the conditions may be made more stringent based on local risk assessment to the laboratory worker, like the compromised immune status of the worker due to diabetes, chronic illness, use of any immunosuppressive drug etc., possibility of transmission via the aerosol route or handling large volumes of cultures etc. These may also be relaxed based on other factors like handling of low pathogenic strains, etc.

Biosafety should form an integral part of the biorisk management of the laboratory. This must include risk assessment by identifying and prioritizing biological materials at the facility and analysis of specific security scenarios for them. Access control, material management and personal security management are features that need to be considered as well as the case for transportation of samples within or outside of the facility. Information security and incident and emergency response planning asks for merging aspects of biosecurity with biosafety.

The important biosafety considerations, commensurate with the BSL-3 containment facilities, for handling the microbe or clinical specimens include the following:

- ✦ Physical containment for personnel
 - Recommended respiratory protection: Filtering face piece (FFP) 3 respiratory protection masks
 - Recommended personal protective equipment: Gloves, safety goggles and protective clothing
- ✦ Best disinfection: 70% ethanol, 2% formaldehyde solution, 1% NaOH, 1% sodium hypochlorite, 5% Calcium hypochlorite and 1% KMNO₄
- ✦ Best decontamination: Disinfectant plus additional formaldehyde fumigation
- ✦ Precautions for packing and shipment of biological samples destined for lab diagnosis

For control and eradication of disease in the country, the disease needs to be notified urgently upon identification. Considering the zoonotic nature of infection, quick diagnosis is of utmost significance. The handling of organisms and clinical specimens must be handled with due care with maximum precautions, as per guidelines. These must be taken into account starting from the collection of samples in the field, testing in lab, handling of organisms for research and handling of affected animal during elimination and disposal of carcass.

24. Biorisk Assessment in Laboratory

Jagadish Hiremath

ICAR-National Institute of Veterinary Epidemiology and Disease Informatics

Number of biohazard agents are handled and processed in different type of laboratory settings at varied levels of number and quantity. Each agent or toxin are dangerous and pose risk to personnel handling in the laboratory. It is responsibility of the laboratories to ensure that needful practices, regulations or procedures are in place for safe handling of biohazard agent and materials. The first step towards this is assessing the biosafety and biosecurity risk present in the laboratory. Biorisk assessment is the process of identifying the laboratory biosafety and biosecurity hazards and their effective management to minimize their potential consequences. Risk assessment acts as decision support system to reduce the risk present in the laboratory. Mitigation of risk ultimately protects the laboratory personnel, the environment and the animal and human communities. The international agencies like WHO, OIE and US Biosafety in Microbiological and Biomedical Laboratories have developed guidance documents on Biorisk management. Risk assessment has to be done periodically as agents, experiments, processes and technologies change so does the risk.

Biorisk assessment is systematic process that reviews all the aspects of work environment, facility location, proposed experimental procedures, laboratory personnel, storage, sample packaging and transportation, decontamination of biohazard waste, access policy and security. Risk assessment can be qualitative or quantitative.

The basic risk assessment involves following process

1. *Define the situation* (What work is occurring?): This step involves activities like hazard identification, host range for the hazard and defining the research activities with laboratory conditions
2. *Define the risks* (What can go wrong?): Every activity identified has associated procedures or hazards/agents to which one can be exposed either inside or outside the laboratory settings. Such points of exposure are to be define and graded and well characterized.
3. *Characterize the risks* (How likely is it to happen? What are the consequences?): Once the risk is identified the exposure is likely with some consequence. Determining how likely the risk can happen and if happens how dangerous the consequences are is important to grade the risk levels and plan management strategies
4. *Determine if the risks are acceptable* (Engage management and other key stakeholders): If the assessed risk is acceptable then risk assessment results should be documented. The unacceptable risks are to be discussed among the stakeholders to determine mitigation measures.
5. *Implement risk mitigation measures* (Ensure all the risks are acceptable post implementation of mitigation measure) The completion of risk assessment process finally identifies and assesses the risk for which the mitigation measures are to be formulated. Among the identified risks, the most important risks are to be targeted for mitigation measures. The risk assessment document, the observations and management actions are to be well documented.

References:

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- o https://orm.uottawa.ca/sites/orm.uottawa.ca/files/biorisk_assessment_process.pdf
- o Biorisk management laboratory biosecurity guidance (September 2006), World Health Organization

Practical

Risk Assessment

Jagadish Hiremath

Introduction:

Biorisk assessment is the process of identifying the laboratory biosafety and biosecurity hazards and their effective management to minimize their potential consequences. Risk assessment of every activity which involves handling biohazard materials or agents has to be done periodically. The risk is dynamic factor which changes with change in material, procedure, personnel, equipment etc.,

Objectives:

1. To demonstrate the process of risk assessment

Activity:

Groups of three will be encouraged to do risk assessment by providing a procedure

25. Laboratory Biosafety

Harshad Murugkar

Principal Scientist and Biosafety officer

National Institute of High Security Animal Diseases

Anand Nagar, Bhopal - 462 021

The risks posed by diagnostic and research laboratories that are involved in handling dangerous infectious pathogens to the personnel working in these laboratories and to the environment and thereby to the larger community are very high. In addition to the specific instances of laboratory-associated infections throughout the history of microbiology, a number of incidents have been recorded in the recent past wherein the laboratories have been responsible for the accidental release of a number of hazardous agents into the environment with disastrous consequences. One of the unique challenges diagnostic laboratories face is the constant flow of clinical samples whose etiology is unknown at the point of their receipt. Such samples predominantly originate from clinically ill cases and are potentially a major biosafety risk for the diagnosticians as well as other workers in the laboratory. The use of biosafety concept as a scientific method for prevention of spread of infection through man, material and environment has been limited to a very few veterinary and medical laboratories in the country. With the growing realization of the threat of the exotic and emerging diseases, biosafety is gaining better administrative and technical attention in the laboratories handling infectious organisms.

Laboratory biosafety

Laboratory biosafety is the term used to describe the containment principles, technologies and practices that are implemented to prevent unintentional exposure to pathogens and toxins, or their accidental release in the environment. A combination of engineering controls, work practices, and personal protective devices are employed at both the primary and secondary biocontainment levels in order to achieve and optimally biosafe environment. Primary containment protects the laboratory workers and the immediate laboratory environment from exposure to biological agents and is achieved through good microbiological techniques and the use of safety equipment and personal protective equipment. Secondary containment protects the environment outside the laboratory, and is provided by facility design and operational procedures. The containment facility of a laboratory is a very crucial component of biosafety principles to prevent the release of infectious agents into the environment. The concept of biosafety levels in the laboratory is primarily based on this concept of containment.

Biocontainment laboratories

The primary objective of a biocontainment laboratory is to ensure that the environment and community is shielded from the risk of contamination with all the pathogens being handled inside the containment laboratory. This is primarily achieved through facility design and engineering controls. Such facilities operate with inward directional air flow; i.e., air must move from the corridor (area of least contamination) into the room where staff members manipulate the animal or animal by-products (area of greatest contamination). Air exhausted from these rooms cannot be re-circulated to other rooms; the air must be discharged to the outside. When there is a very high potential for aerosol generation, additional facility related engineering controls such as sealed penetrations through the walls, double door access to the rooms, or installation of HEPA filters in the exhaust air system may be necessary. A facility can also apply engineering controls locally to reduce aerosol spread. Adequate controls are also required to be put in place to ensure that no material (whether solid or liquid) leaves the containment laboratory without adequate decontamination. Strict access control inside the biocontainment laboratory for only the personnel trained on biosafety aspects involved in the laboratory is a must to prevent accidental transmission of infectious material outside the containment laboratory.

Risk Assessment for determination of bio-containment level of a laboratory

Risk assessment is a critical step in the selection of an appropriate containment level for the microbiological work to be carried out. A detailed local risk assessment should be conducted to determine whether work requires containment level 1, 2, 3 or 4 facilities and operational practices. In addition to the risk group classifications, which are based on the risk factors inherent to the organism, the other factors associated with laboratory operation that should be examined include potential for aerosol generation, quantity of material and concentration of the agent including its stability in the environment (inherent biological decay rate). The type of work proposed (e.g., *in vitro*, *in vivo*, aerosol challenge studies) and the use of recombinant organisms also influence the risk assessment. In addition to the inherent characteristics of each organism, the containment system includes the engineering, operational, technical and physical requirements for manipulating a particular pathogen.

Laboratory practices and techniques

Good laboratory practices are the key to safe working atmosphere within the laboratory premises. Biocontainment laboratories demand a high level of work discipline and ethics to ensure that an individual worker is not responsible for spreading the infection throughout the laboratory premises. Hand washing is an important means of preventing the spread of infectious contaminant. Workers should wash their hands after removing gloves and before leaving the laboratory, procedure room, or animal room. Liquid soap dispensers are preferable to soap bars to minimize cross contamination. After thorough washing, workers should dry their hands with disposable paper towels. Eating, drinking, smoking, handling contact lenses, and applying cosmetics should not be permitted in laboratories, nor should any other activities that might involve hand-to-mouth or hand-to-eye contact, such as mouth pipetting. Staff members should perform all manipulations of potentially infectious materials with great care so as to minimize aerosol production. The personnel should use special devices such as sealable centrifuge cups, blenders, or homogenizers whenever there is a high potential for creating aerosols of infectious microorganisms. Workers must also rely on personal protective devices, such as respirators, to minimize their exposure to infectious aerosols when containment devices offer insufficient protection. The person who generates infectious waste or contaminates equipment, work surfaces, or other areas is responsible for decontamination before the next person begins work. Chemical disinfection or, preferably, steam autoclaving, is recommended for decontaminating reusable materials before washing. When finished working, staff members must dispose of contaminated waste materials, and package them according to local infectious waste regulations prior to disposal.

Safety equipment

Many procedures in the laboratory such as centrifuging, mixing, and pipetting that involve high energy movement of the material tend to produce respirable aerosols that stay airborne for extended periods and are small enough to be inhaled. Low energy procedures including opening containers and streaking plates produce droplets that settle quickly on surfaces, skin, and mucous membranes. All these procedures increase the exposure risk to the workers and may result in serious laboratory association infections. Safety equipment includes biological safety equipment, safety centrifuge cups, and other engineered controls designed to minimize exposure to biological agents. Biological safety cabinets (BSCs) are the most important safety equipment for protection of personnel and the laboratory environment, and most BSCs also provide product protection. Safety equipment is most effective at minimizing exposure when workers are trained in its proper use and the equipment is regularly inspected and maintained.

Personal protective equipment (PPE)

Laboratory workers can protect against splashes and splatters by adhering to careful work practices and rigorous use of personal protective devices. Face shields provide protection for eye and mucus membranes. Lab coats or work uniforms will help prevent contamination of street clothes and should be changed whenever visibly soiled. Staff members should autoclave lab coats before disposal or laundering; soiled

labcoats should go to on site or professional cleaners only. Latex or vinyl gloves provide barrier protection for hands. Staff must change gloves that are torn or visibly contaminated, and should autoclave them before disposal. Gloves and other protective devices cannot prevent needle sticks or other unintentional injuries caused by sharp instruments, broken glass, etc. Self sheathing needles are available, as are other engineered safety devices. Needles must not be bent, cut, or recapped: they must be discarded directly into puncture resistant and leak proof containers

Transport of infectious agents

The transportation of infectious substances is an essential part of routine laboratory procedures in both research and diagnostic settings. It is essential to use appropriate packaging material and method that withstands rough handling and prevents the leakage of liquid infectious material to the outside. There should be appropriate documentation and labeling of the package with the biohazard symbol to alert the workers in the transportation chain about the hazardous contents of the package. Appropriate surface decontamination procedure should be followed when the package is being transported. Training of workers in the transportation chain is very important to familiarize them with the hazardous contents so as to be able to respond to emergency situations.

Decontamination and Disposal of bio-hazardous wastes

A large quantity of potentially dangerous biohazardous waste is generated in the diagnostic laboratories which can potentially cause harm not only to the workers inside the laboratory but also may lead to the contamination of the environment if it is disposed off without any pretreatment and decontamination. Complete decontamination and safe disposal of biohazardous waste is a crucial biosafety requirement of a bio-containment laboratory. The level of decontamination and waste disposal facility required to handle the bio-waste depends on the biosafety level of the containment laboratory, type of waste being generated, risk group to which the infectious agent belongs, research agenda of the laboratory and the local environmental rules and regulations.

Health and medical surveillance

In order to ensure that the laboratory personnel themselves do not become the carriers of the infections to outside the laboratory, a health and medical surveillance programme (including pre-employment and then periodic testing) for the individuals working in the laboratory needs to be in place. Such programmes should be consonant to the kind of diagnostic work carried out in the laboratory and the type of agents being handled. This programme may include a medical examination; serum screening, testing and/or storage; immunizations; and possibly other tests as determined by the risk assessment process.

Training

All persons who work in a laboratory bear responsibility to minimize risk of infection through consistent good safe microbiological practices and procedure. All at-risk persons working in a facility should receive appropriate training on that facility's particular biohazards, precautions, and biohazard evaluation procedures. Personnel should receive annual updates and additional training when procedures or policies change. Laboratory workers and animal care personnel should know how to recognize hazard warning signs, to protect themselves and their coworkers against each hazard, and to react properly in the event of emergencies, such as an unintentional biohazard material release. Training should be appropriate for the employee's education, experience, and language skills, and should be performance based to ensure that employees master the skills before encountering a hazard. The facility should incorporate the syllabus of the training programs into its safety manual. Supervisors should assess each employee's biosafety knowledge during the formal training period and later through subsequent regular observation of routine activities.

Resource management in biocontainment laboratories:

Biocontainment laboratories are highly specialized facilities and requires high level of commitment in terms of availability of trained human resources and capital for maintenance and regular validation of these laboratories. Before committing to construction of a biocontainment laboratory, proper planning should go into the future requirements in terms of availability of biosafety specialists both for laboratory biosafety management and for the biosafety engineering involving the biocontainment facility. Proper financial resource planning is also required to be put in place in order to meet the recurring biosafety consumable costs and the biocontainment facility maintenance cost such as validation and testing of HEPA filters in the HVAC system or containment equipment such as biosafety cabinets. A regular certification and validation plan has to be designed to ensure prompt fault detection and rectification mechanism in the biocontainment facility. Moreover, adequate back up system to prevent the failure of key biocontainment equipment for e.g., electricity, autoclaves, sterilizers, etc. is crucial to ensure that no infectious pathogen escapes the biocontainment facility owing to the failure of the containment devices.

In conclusion, biocontainment laboratories are becoming key facilities in ensuring a safe working premises that protects not only the workers inside the laboratory but also the general community by preventing the unintentional escape of infectious agents into the environment. This however demands a high level of commitment right at the level of the topmost administrator involved in laboratory administration to the lowest level of laboratory worker.

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26. Abbreviations

ABBREVIATIONS	
ABSL	Animal Biosafety Laboratory
AHU	Air Handling Unit
BBP	Blood Borne Pathogen
BMS	Building Management System
BSC	Biosafety Cabinet
BSL	Biosafety laboratory
BSO	Biosafety Officer
CDC	Center for Disease Control
cfm	Cubic Flow per Minute
DBT	Department of Biotechnology
ETP	Effluent Treatment Plant
FAO	Food and Agricultural Organization
GLP	Good Laboratory Practices
GMO	Genetically Modified Organism
GMT	Good Microbiological Techniques
HEPA	High Efficiency Particulate Air
HVAC	Heating Ventilation and Air-Conditioning
IBSC	Institute Biosafety Committee
OIE	World Animal Health Organization
Pa	Pascal (SI unit of Pressure)
PPE	Personal Protective Equipment
RH	Relative Humidity
SOP	Standard Operating Procedure
ULPA	Ultra-Low Penetration Air
VBM	Valuable Biological Material
WHO	World Health Organization

27. Glossary

Biohazard	Biohazards are infectious agents or hazardous biological materials that present a risk or potential risk to the health of humans, animals, or the environment.
Biohazardous waste	Any liquid or solid waste generated through the handling of specimens from humans or animals that may contain infectious agents. Cultures of infectious agents, human anatomical remains, and animal carcasses that may be infectious are also considered biohazardous waste.
Biological materials	Any materials containing genetic information and capable of reproducing itself or being reproduced in a biological system. Biological materials microorganisms, Recombinant DNA (rDNA), Cell lines, Animals (live or tissues and biological fluids), Plants, Human tissue or biological fluids, Microbial Toxins. (http://www.ncat.edu/research/dored/biosafety-guide.html)
Biosafety	Biosafety is the application of safety precautions that reduce a laboratorian’s risk of exposure to a potentially infectious microbe and limit contamination of the work environment and, ultimately, the community. (http://www.cdc.gov/training/QuickLearns/biosafety/)
Biosafety Officer	The Biosafety Officer is the designated officer appointed in case rDNA research involves biosafety level 3 or 4 containment facilities or large scale rDNA research.
Biosecurity	The term “biosecurity” has multiple definitions. In the animal industry, the term biosecurity relates to the protection of an animal colony from microbial contamination. The term refers to the protection of microbial agents from loss, theft, diversion or intentional misuse. This is consistent with current WHO and American Biological Safety Association (ABSA) usage of this term.
BMS	Are computer-based systems that help to manage, control and monitor building technical services.
Containment	Describe safe methods for managing infectious agents and/or regulated GMOs/LMOs/rDNA material in the laboratory environment where they are being handled or maintained.
Decontamination	Process to remove, inactivate, or destroy bloodborne or other pathogens on a surface or item, to the point where they are no longer capable of transmitting infectious particles, and the surface or item is rendered safe for handling, use, or disposal.
Disinfection	A physical or chemical means of killing microorganisms, but not necessarily their spores.


Dry heat sterilization	a method of sterilization that uses heated dry air at a temperature of 320° to 356° F (160° to 180° C) for 90 minutes to 3 hours.
GMO	Organisms (i.e. plants, animals or microorganisms) in which the genetic material (DNA) has been altered in a way that does not occur naturally by mating and/or natural recombination. (WHO)
HVAC	Heating, ventilation, and airconditioning (HVAC) systems for Research Laboratories and Animal Facilities shall be designed to maintain the space temperature and humidity at the required set point. These systems shall automatically adjust, as necessary, to respond to varying space cooling demands in laboratories and animal facilities. Air-change rates, temperature and humidity shall be closely monitored and controlled on a continuous basis. The System shall provide adequate ventilation to remove fumes, odors, airborne contaminants, and to safely operate fume hoods continuously.
IBSC: The Institutional Biosafety Committee (IBSC)	IBSC is a statutory committee of an organization undertaking rDNA activities, constituted as per provisions of Rules, 1989 and chaired by the Head of the organization or his designate (a suitable senior officer).
Infectious agent	Is any microorganism, bacteria, mold, parasite, or virus that normally causes or significantly contributes to increased human mortality. Infectious agents have also been defined as any materials that contains an organism capable of being communicated by invading and multiplying in body tissues
Laboratory biosecurity	Laboratory biosecurity refers to institutional and personal security measures designed to prevent the loss, theft, misuse, diversion or intentional release of pathogens and toxins. (WHO Laboratory Biosafety Manual, 3rd edition, 2004)
Physical containment	Structures and procedures designed to prevent the release of viable organisms into the environment.
PPE (Personal protective equipment)	Refers to protective clothing, helmets, goggles, or other garments or equipment designed to protect the wearer's body from injury or infection. The hazards addressed by protective equipment include physical, electrical, heat, chemicals, biohazards, and airborne particulate matter. Protective equipment may be worn for job-related occupational safety and health purposes.
Relative humidity	Is a ratio, expressed in percent, of the amount of atmospheric moisture present relative to the amount that would be present if the air were saturated. Since the latter amount is dependent on temperature, relative humidity is a function of both moisture content and temperature. Relative Humidity is derived from the associated Temperature and Dew Point for the indicated hour.

Risk assessment	Assessing the hazards posed by the biological agent and the risks of associated laboratory activities.
Risk group	Biological agent posing possible threat which is grouped using three criteria: Pathogenicity, or the ability to cause disease in humans or animals; Availability of medical countermeasure or prophylactic treatment for the associated infection; and, Ability of the disease to spread.
Sanitization	Sterilization describes a process that destroys or eliminates all forms of microbial life and is carried out in health-care facilities by physical or chemical methods. (https://www.cdc.gov/hicpac/Disinfection_Sterilization/1_sumIntroMethTerms.html)
Sharp waste	Devices or objects capable of cutting or piercing, such as hypodermic needles, razor blades, and broken glass.
Sterilization	A process that destroys and/or removes all classes of microorganisms and their spores. (WHO)
Wet/ moist heat sterilization	Exposure of microorganisms to saturated steam under pressure [15 minutes at 121-124 °C (200 kPa)] in an autoclave achieves their destruction by the irreversible denaturation of enzymes and structural proteins.


28. SAARC Regional Training - 2019

A. Trainee Nominees



	MS.FAHIMA MAHBOBI	Member of Serology,Central Veterinary Diagnostic Laboratory, Animal Health Directorate, Darulaman Street-Senaturiam, Kabul, Afghanistan
	MS.SORAYA RAFA	Member of Teaching Laboratory, Central Veterinary DiagnsoticLaboratory,Animal Health Directorate,Darulaman Street-Senaturiam, Kabul, Afghanistan
	MR. HAMIDULLAH THUSSAINKHIL	
	DR.MD.MUNIRUZZAMAN	Scientist Officer,EDIL,Department of Livestock Services Joypurhat,Bangladesh
	DR.MD.ZAKIR HASSAN	Scientist Officer, Bangladesh Livestock Research Institute,Savar,Dhaka,
	MR.MIGMA	Senior Laboratory Technician,National Centre for Animal Health,Department of Livestock,Thimpu,Bhutan Email: migma88@yahoo.com
	MR.SANGAY TENZIN	Senior Laboratory Technician,Regional Livestock Development Centre,Department of Livestock,Wangdi,Bhutan
	DR. ARCHANA M	Southern Regional Disease Diagnostic Laboratory,Bangalore.
	DR.KALPANA DUSHYANT MUGLIKAR	Western Regional Disease Diagnostic Laboratory,Pune ,Govt .of Maharashtra.
	DR.BALARAM THAPA CHHETRI	Chief, Central Referral Veterinary Hospital
	MRS.NAJUMA JOSHI	Livestock Development Officer,National Animal Feed and Livestock,Quality Management Laboratory

	DR.L.D.KITHSIRI	Director, Public Health Veterinary Service, Sri Lanka.
	MS.R.D.S.S.WIJESEKARA	Programme Assistant, Sri Lanka School of Agriculture, Sri Lanka.
	DR.H.KOTHALAWALA	Veterinary Research Officer, Veterinary Research Institute Gannoruwa, Sri Lanka,

B. Organizing Team - SAC

	Dr. S. M. Bokhtiar	Director, SAARC Agriculture Centre BARC Complex, New Airport Road, Farmgate, Dhaka, Bangladesh
	Dr. Ashis Kumar Samanta,	FNAVS, FSAB Post Doctorate (Australia) Senior Programme Specialist (Livestock) SAARC Agriculture Centre BARC Complex, Farmgate, Dhaka 1215 Bangladesh

C. Organizing Team - ICAR-NIVEDI

 	Dr. Parimal Roy	Director, ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Yelahanka, Bengaluru, India
	Dr. Jagadish Hiremath	Senior Scientist & Biosafety Officer ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Yelahanka, Bengaluru, India
	Dr. R. Sridevi	Scientist ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Yelahanka, Bengaluru, India
	Dr. G. B. Manjunatha Reddy	Scientist ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Yelahanka, Bengaluru, India
	Dr. Siju Susan Jacob	Scientist ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Yelahanka, Bengaluru, India



**ICAR-National Institute of Veterinary Epidemiology
and Disease Informatics (NIVEDI),**

Post Box No.6450, Ramagondanahalli, Yelahanka, Bengaluru-560064, Karnataka, India.

Ph: +91-80-23093110, 23093111, Fax: +91-80-23093222

Website: www.nivedi.res.in, Email: director.nivedi@icar.gov.in