Biosecurity and biosafety in the laboratory: Experience of the OIE Reference Laboratory for Avian Influenza

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VIROLOGY JOURNAL

RESEARCH Open Access

Aerosolized avian influenza virus by laboratory manipulations

Conjunctivitis in Humans Exposed to Infected Seals

During postmortem studies on the stranded and dead seals, four individuals

A case report of fowl plague keratoconjunctivitis

H. R. TAYLOR
From the Department of Ophthalmology, University of Melbourne, Royal Victorian Eye and Ear Hospital, Melbourne, Victoria

A. J. TURNER
From the Division of Veterinary Public Health, Department of Agriculture, Melbourne, Victoria

SUMMARY A case of human fowl plague keratoconjunctivitis occurred after accidental laboratory exposure. The conjunctivitis was characterised by follicle formation and a mucopurulent discharge, and ran a self-limiting course over two weeks. The keratitis was of an unusual type and consisted of small intraepithelial opacities, which appeared after one week and resolved completely over the next three weeks. The infection, confirmed by viral culture, was produced by Dutch strain (Hav 1 Neq 1) of fowl plague virus.

The possibility that an influenza infection in humans caused by avian influenza viruses A viruses could occur following a laboratory accident is a risk to which it is crucial to be constantly alert

Which is the first thing you have to use before handling any infectious biological agent?

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Do not follow just your instinct!!

Risk Assessment

Risk Assessment depends on a coordinated approach that enables the appropriate selection of measures to ensure reasonable and adequate laboratory security without unduly affecting the scientific work

*"The most important component of **risk assessment** is **professional judgment**.*

*Risk assessments should be performed by the individuals most familiar with the specific characteristics of the **organisms** being considered for use, the **equipment** and **procedures** to be employed, **animal models** that may be used, and the **containment equipment and facilities** available."*

WHO Laboratory Biosafety Manual, 3rd Edition (2004)

Remember….

- Protecting the **public's health**
- Protecting **employees / co-workers**
- Protecting **research** at the institution
- Protecting the **image** of the institution

Risk Assessment

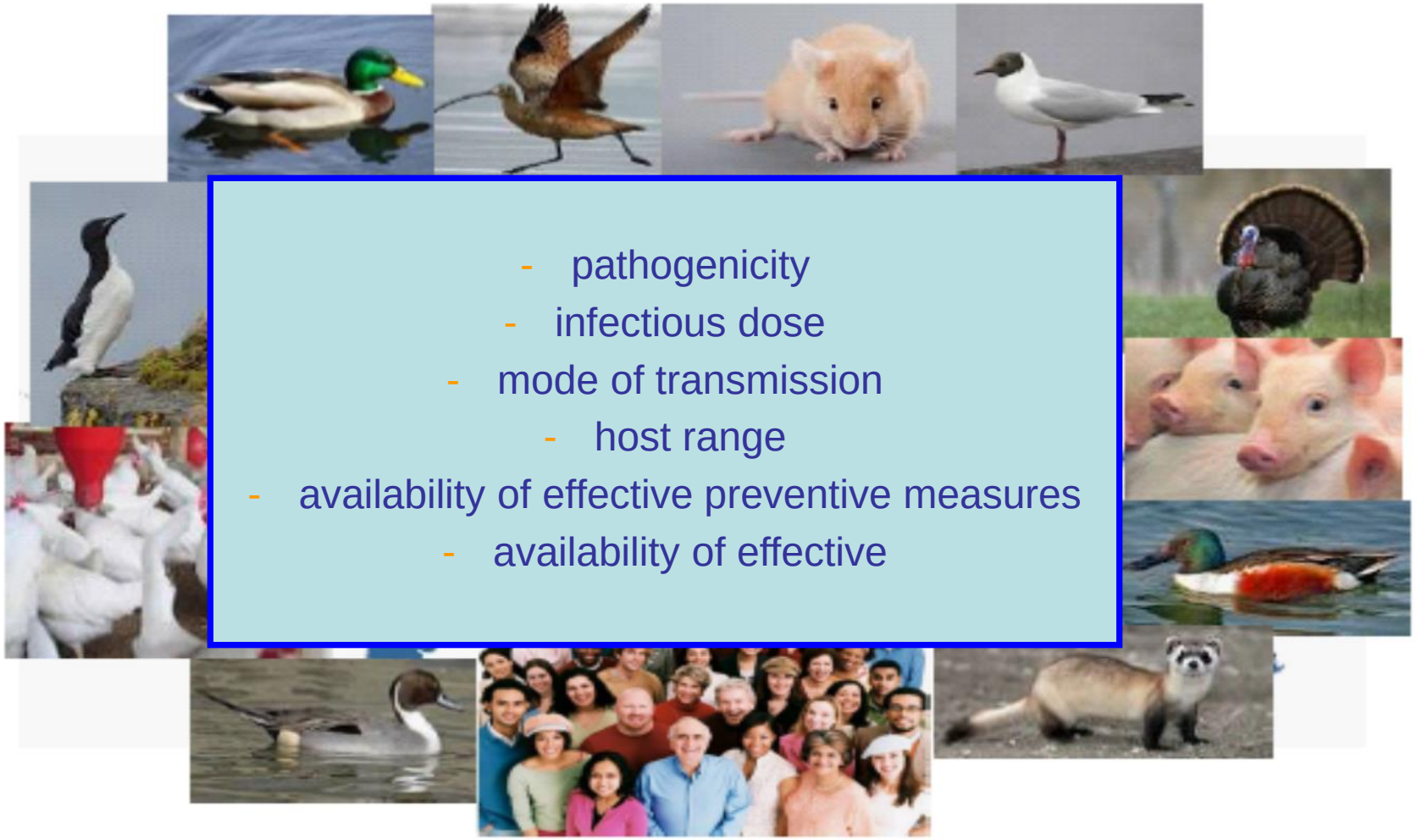
Primary factors to consider:

1. Agent hazards (Risk Group)

2. Laboratory procedure hazards and hazards associated with work practices

Hazardous Characteristics of Avian Influenza viruses

The World Health Organization (WHO) has recommended an agent risk group classification for laboratory use based on these principal characteristics:



- pathogenicity
- infectious dose
- mode of transmission
- host range
- availability of effective preventive measures
- availability of effective

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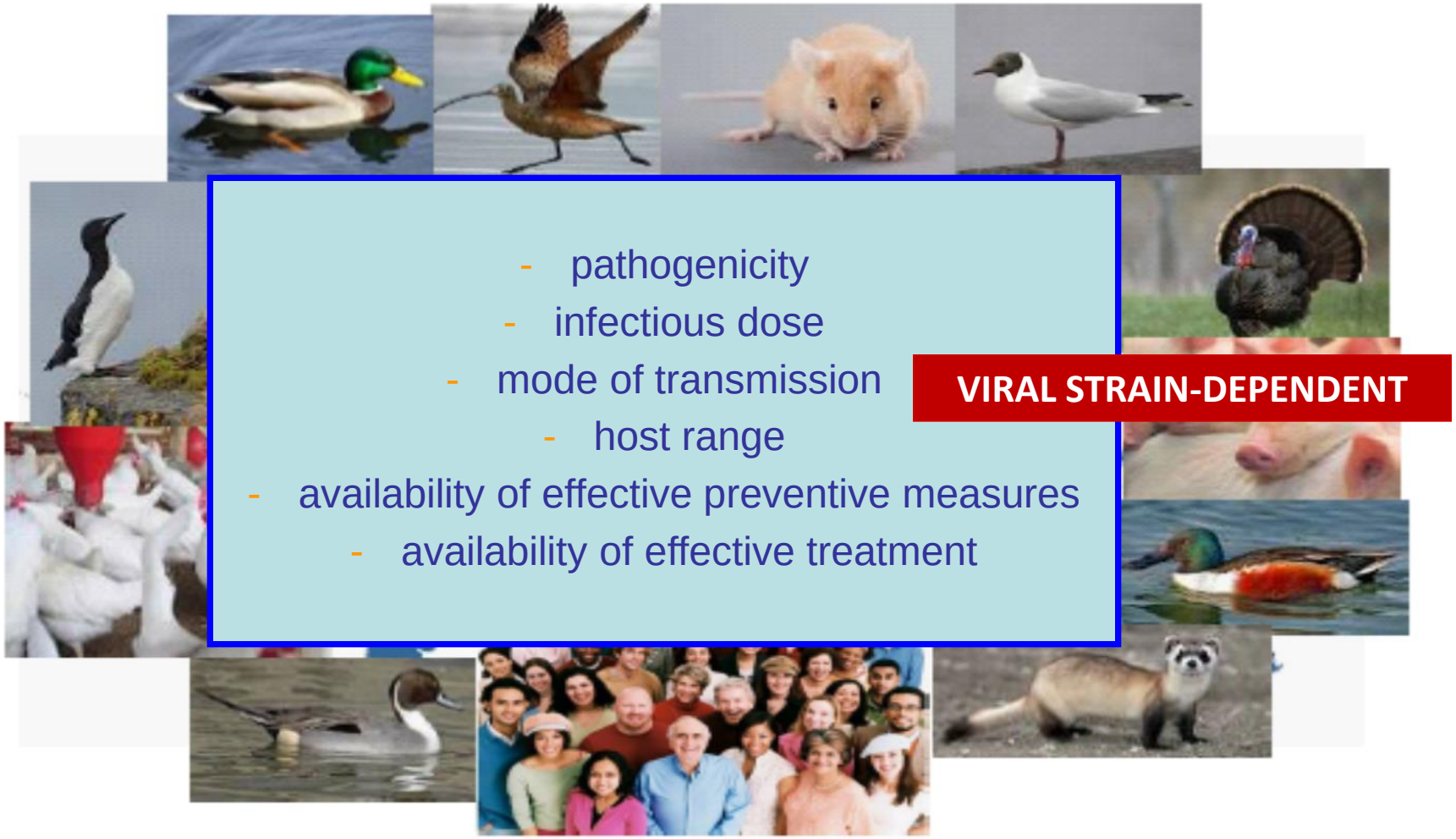
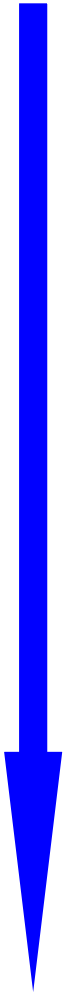


- pathogenicity
- infectious dose
- mode of transmission
- host range
- availability of effective preventive measures
- availability of effective treatment

VIRAL STRAIN-DEPENDENT

RISK GROUP CLASSIFICATION WHO, 2004 *(FOR LABORATORY USE ONLY)*	**DESCRIPTION**
Risk Group 1 (no or low individual and community risk)	A microorganism that is unlikely to cause human or animal disease.
Risk Group 2 (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 (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 (high individual and community risk)	A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive **measures are not usually available**.



Increasing risk to the laboratory worker and the community

(WHO may differ from OIE class)

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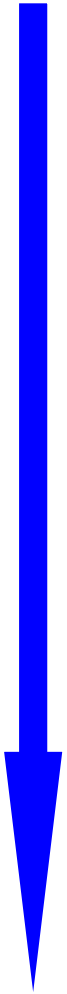

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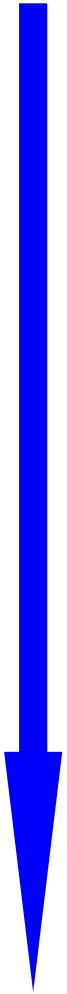
Risk Group 3 (high individual risk, low community risk) **Higher level containment being indicated for H5/H7 LPAI and HPAI viruses (OIE, Terrestrial Manual 2015)**	A pathogen that **usually causes serious human or animal disease but does not ordinarily spread** from one infected individual to another. Effective treatment and preventive measures are available.
Risk Group 4 (high individual and community risk)	A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.



Risk Assessment

Primary factors to consider:

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Hazardous Characteristics of Laboratory Procedures

Routes of transmission in the laboratory for avian influenza virus:

1. Direct skin, eye or mucosal membrane exposure
2. Parenteral inoculation (syringe needle, other contaminated sharp or bites from infected animals)
3. Ingestion (e.g. hand to mouth exposure)
4. Inhalation of infectious aerosols

The first three routes of laboratory transmission are easy to detect (20% of all reported Laboratory-Associate Infections LAIs)

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Biosafety Levels (BSLs)

Four Biosafety Levels (BSL1 to 4) are designated in ascending order, by degree of protection provided to personnel, the environment and the community.

Biosafety levels (BSLs)

BSLs consist of a combination of:

- **laboratory practices and techniques** (the most important element of containment is strict adherence to standard microbiological practices and techniques)
- **safety equipment** (primary barriers and personal protective equipment-PPE)
- **laboratory facilities design and construction** (secondary barriers)

Laboratory safety measures for Avian Influenza

RISK GROUP	BIOSAFETY LEVEL	LABORATORY TYPE	LABORATORY PRACTICES	SAFETY EQUIPMENT
1	**Basic - BSL1**	Basic teaching Research	Good microbiological techniques (GMT)	None, open bench work
2	**Basic - BSL2**	Primary health services Diagnostic services Research	GMT, biohazard sign, limited access	Open bench plus BSC (Biological Safety Cabinet) for potential aerosols, autoclave available
3 **2** (high volumes /high conc.)	**Containment - BSL3**	Special diagnostic services Research	As level 2 plus special clothing, controlled access, directional airflow, decontamination of all waste	BSC and/or other primary devices for all activities, environmental and functional isolation, autoclave on site, exhausted air not recirculated
4	**Maximum containment - BSL4**	Dangerous pathogen unit	As level 3 plus airlock entry, Clothing change before entering, shower on exit, special waste disposal (all material decont.)	Class III BSC, or positive pressure suits in conjunction with Class II BSCs, double ended autoclave (through the wall), filtered air, environmental and functional isolation

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1	**Basic - BSL1**	Basic teaching Research	Good microbiological techniques (GMT)	None, open bench work
2	**Basic - BSL2**	Primary health services Diagnostic services Research	GMT, biohazard sign, limited access	Open bench plus BSC (Biological Safety Cabinet) for potential aerosols, autoclave available
3 **2** (high volumes /high conc.)	**Containment - BSL3**	Special diagnostic services Research	As level 2 plus special clothing, controlled access, directional airflow, decontamination of all waste	BSC and/or other primary devices for all activities, environmental and functional isolation, autoclave on site, exhausted air not recirculated

The orthomyxoviruses are risk group 2 agents.

However, the measures required may vary among the subtypes, with higher level containment (e.g. Risk group 3 or 4) being indicated for H5/H7 LPAI and HPAI viruses (Terrestrial manual, OIE).

The primary laboratory hazard is inhalation of virus from aerosols generated by manipulating virus-infected samples. In addition, laboratory infection can result from direct inoculation of mucus membranes through virus-contaminated gloves.

Avian Influenza outbreak and problems encountered by a diagnostic laboratory

- Alert and delivery of samples on unusual time (extra working hours, week-ends, holidays)
- Sudden increase in sample testing (up to several hundreds/week)
- Pressure for faster turn-around-time (TAT)

In combination with

- High quality test performances
- Cost effectiveness

H7N7 HPAI – Italy 2013 Index case (Laying hen- Industrial farm)

H7N7 HPAI – Italy 2013 Index case (Laying hen- Industrial farm)

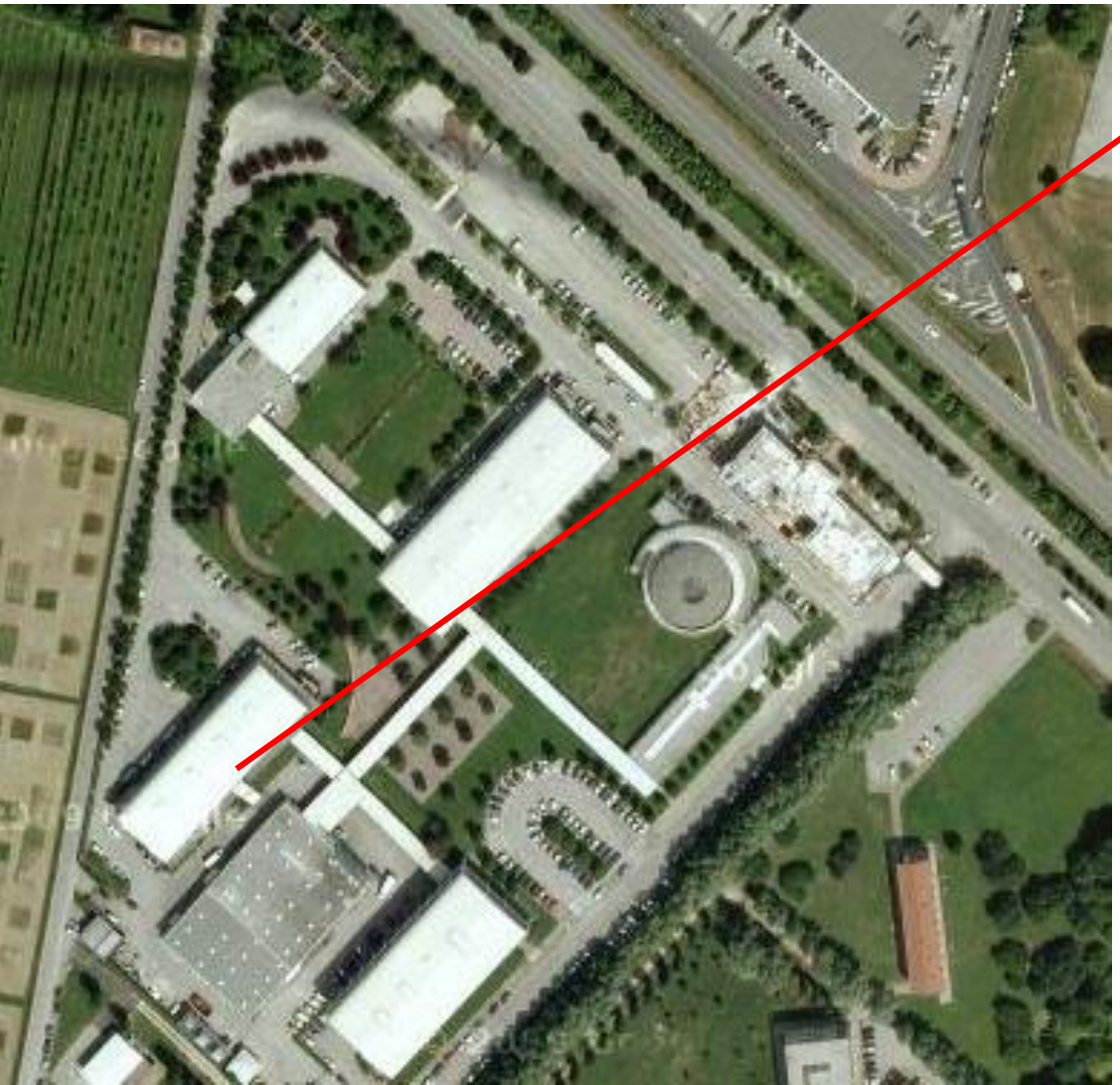
- **13 August** – afternoon: alert (on 12 August a peak of 1188 dead birds out of a total of 23,700 hens was registered)

Assess the risk and identify the BSL

1. Risk group???

2. Biosafety level???

Strutture BSL3 dell'Istituto



BSL3 laboratory

Biosafety actions (BSL3):

1. Supervisor should immediately provide guidelines for safe packaging of the potentially infectious materials **(basic triple packaging system)**

2. Alert the BSL3 supervisor (if different from the person possessing the information related to the outbreak)

To be continued

Biosafety actions (BSL3):

3. Supervisor: decide who enter (at least two persons)

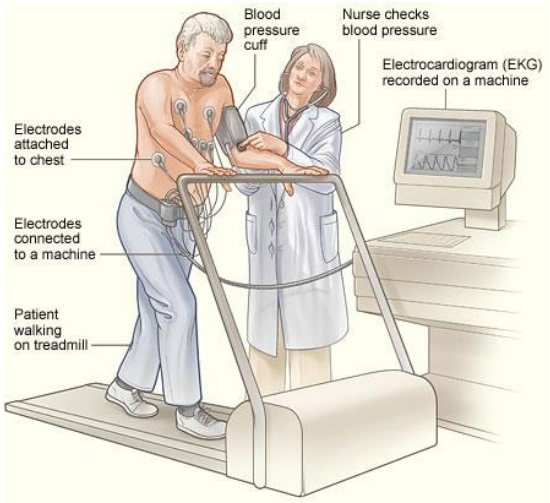
4. All persons handling the samples must be advised of the potential hazards and meet specific entry/exit requirements

5. Only laboratory personnel who received specific **training** in handling pathogenic and potentially lethal agents can enter the BSL3 facilities

To be continued

Laboratory staff must be fully trained in the:

- Handling of pathogens
- Use of safety equipment
- Disposal techniques
- Handling of contaminated waste
- Emergency response



Health check up for the lab staff

H7N7 HPAI – Italy 2013 Index case (Laying hen- Industrial farm)

13 August – afternoon: alert (on 12 August a peak of 1188 dead birds out of a total of 23,700 hens was registered)

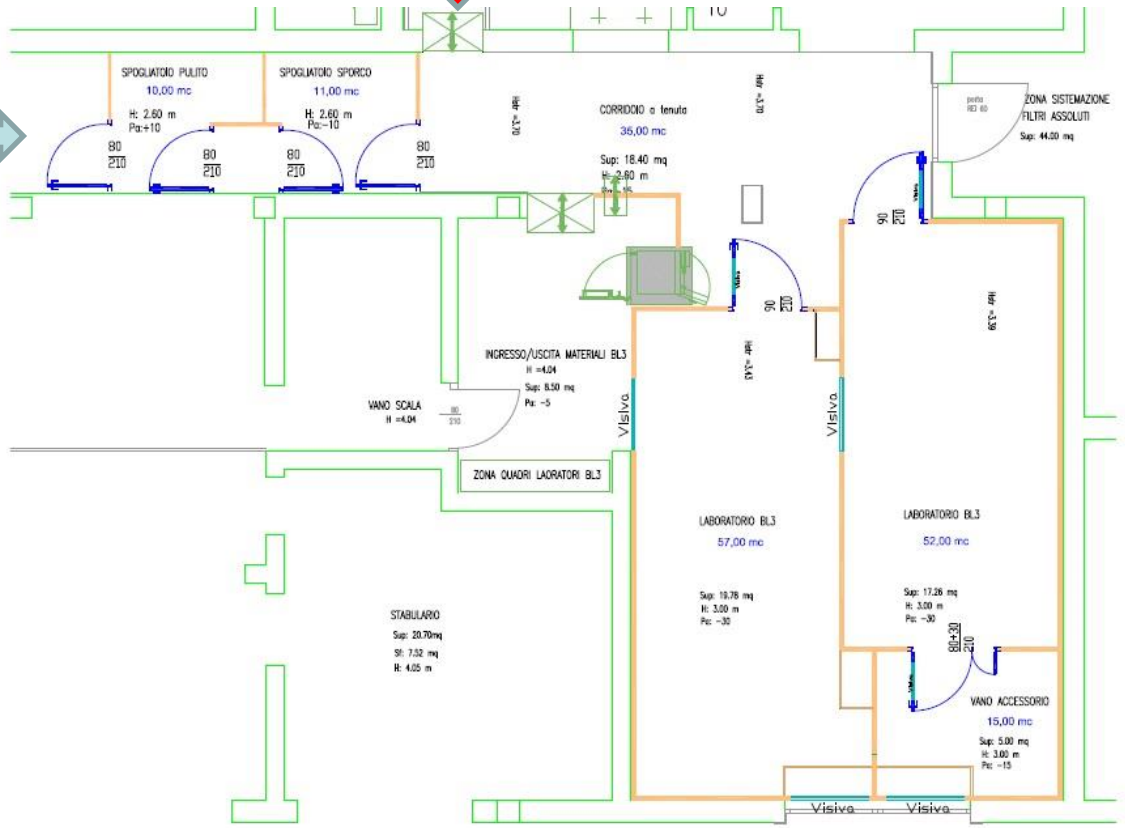
14 August tracheal and cloacal swabs sent to the Reference Laboratory (RL).

14 August – 8:30am. Sample identification and registration at the RL**.**

Biosafety actions (BSL3):

6. Identified and registered potentially infectious materials must be placed in a durable, leak proof container and passed in BSL3 through a pass-box

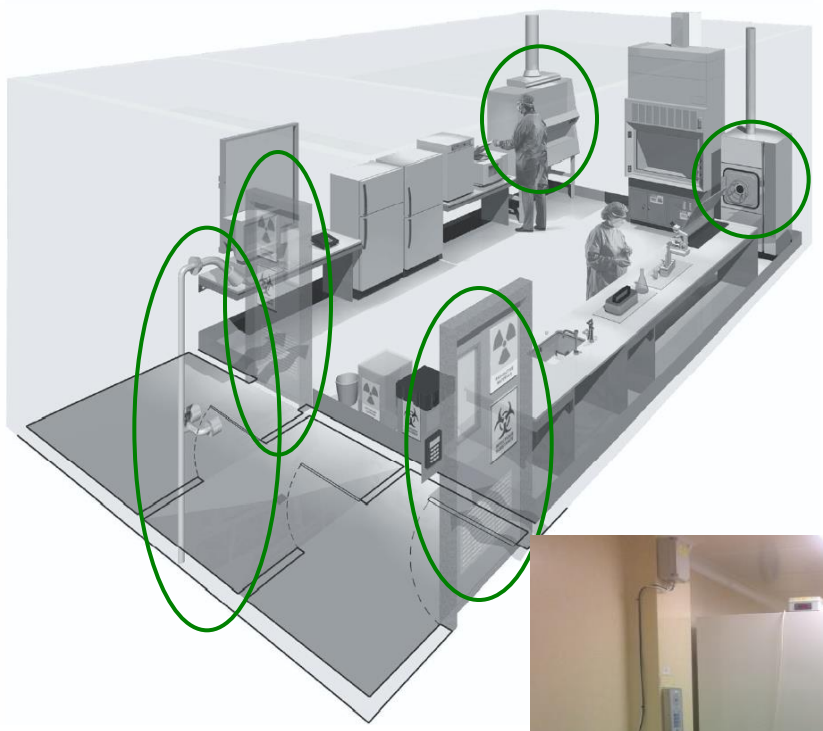
7. BSL3 authorized personnel: restricted access to the laboratory is through two self-closing doors



To be continued

BSL3 equipment

1. Negative air pressure, HEPA filtration, no recirculation
2. Autoclave and BSC
3. Personal Protection Equipment (PPE):head covers, footwear, gloves and appropriate respiratory protection
4. Double door entry and body shower
5. Biohazard sign
6. Emergency tools
7. Storage facilities



Biosafety actions (BSL3):

8. BSL3 personnel apply following standard and special safety practices:

-Wear protective laboratory clothing

To be continued

Principal aspects for PPE management:

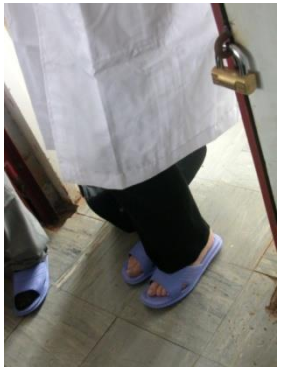
- **Appropriate selection and training on proper use**
- **Cleaning, maintenance, repair**
- **Storage**
- **Individual needs**

?

?

And don’t forget laboratory shoes!

?



No sandals or open-toed shoes in the BSL-2 (or any) laboratory.

Appropriate footwear.

Personal Protective Equipment (PPE)

- Disposable tyvek overall with hood
- Resistant shoe-covers
- Rubber boots
- Disposable gloves (2 pairs)
- Protective glasses
- Respiratory masks with aspiration valve
- Full face mask with P3 or HEPA filter

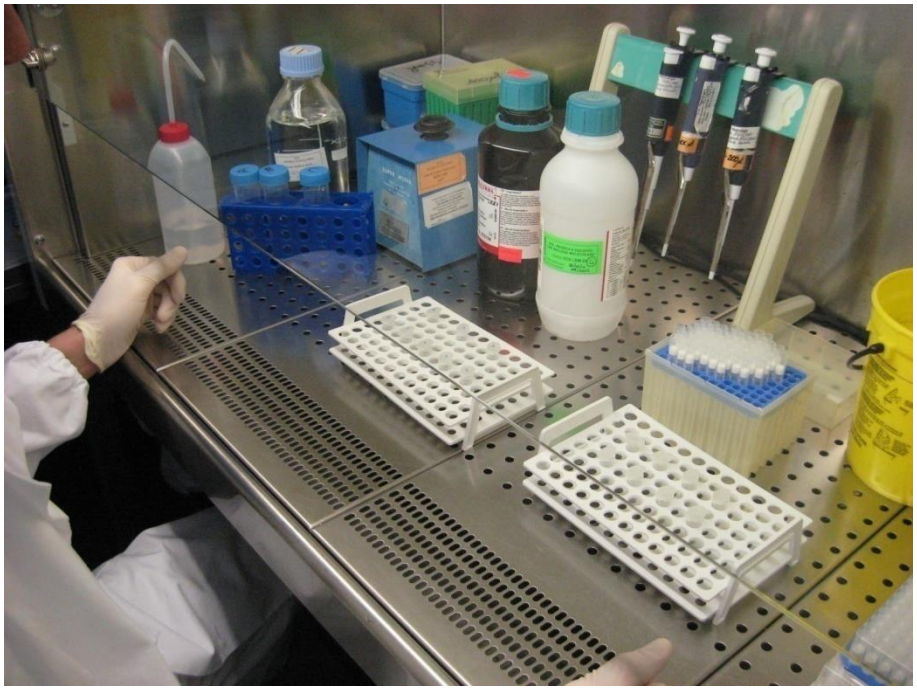
Biosafety actions (BSL3):

9. Potentially infectious materials collected from the passbox must be handled within a BSC II or III. The trained staff proceed with extraction of RNA from the HPAI suspected samples.

To be continued

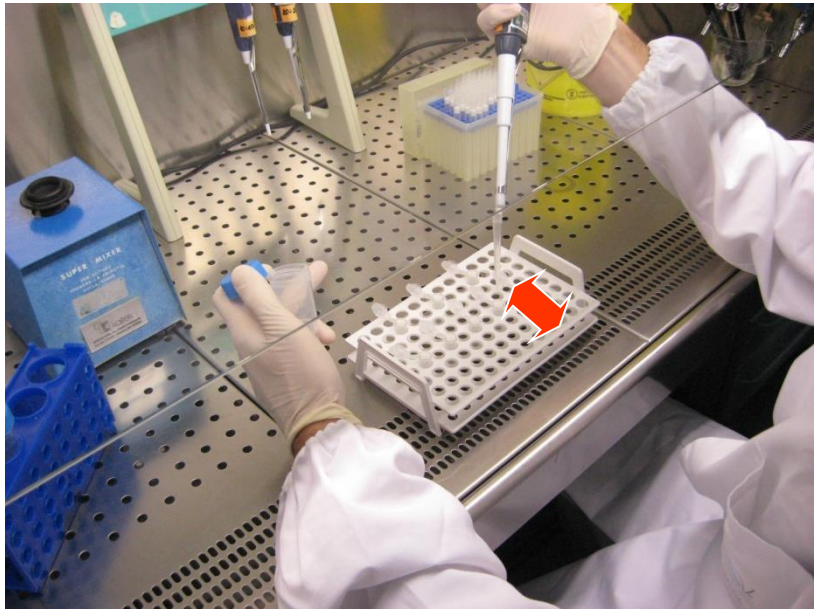
Under the Cabinet

- Do not place too many things under the Biosafety Cabinet!

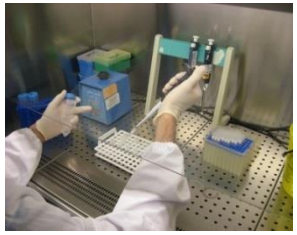
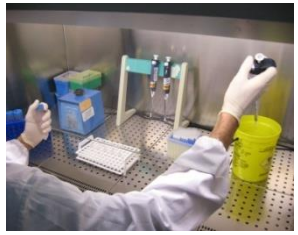
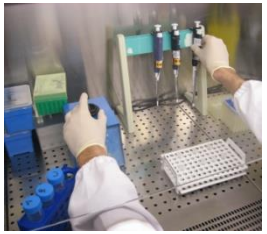
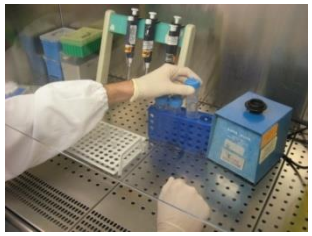


Under the Cabinet

Work at least 10 cm inside the Biosafety Cabinet



- A good organization of both working material and equipment under the Biosafety Cabinet can increase the safety for the operator and reduce the risk of contamination

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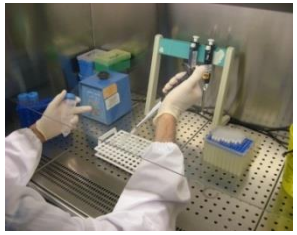
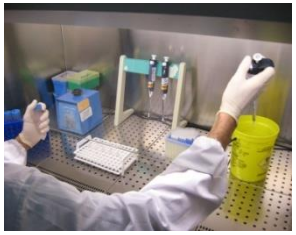
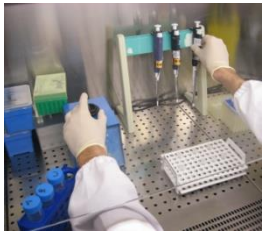





Biosafety actions (BSL3):

10. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.

11. Tubes containing extracted RNA removed from the facility must be appropriately decontaminated and moved outside the BSL3 building using passbox

To be continued

12. Remove PPE in the following order:

1. GLOVES 1° PAIR
2. OVERALL
3. GOGGLES
4. FACE MASK
5. BOOTS
6. GLOVES 2° PAIR

Disposable PPE must be properly discarded (sealed plastic bags). Reusable or non disposal PPE should be cleaned and disinfected in suitable way

COMPLETE HYGIENE MUST BE PERFORMED AFTER REMOVING PPE

HPAI Workflow

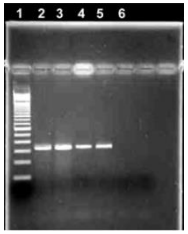
HPAI sample

BSL3

Viral Growth (eggs or cells)

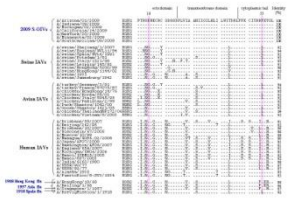
RNA extraction

PCR



BSL2

Sequencing



LPAI Workflow

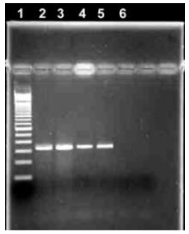
BSL2

LPAI sample

Viral Growth (eggs or cells)

RNA extraction

PCR



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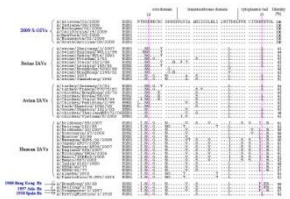

Health considerations

Annual vaccination with influenza human vaccine is recommended to the staff that is exposed to the infection risk to avoid recombination between avian and human virus

Possibility to use antivirus medicines depending on the risk of exposure. The doctor in charge will have to provide to the people under infection risk, instructions about the possible use of the antivirus medicines, both for preventive and therapeutic purpose

Personnel must communicate as soon as presenting any influenza like symptoms to the doctor in charge

Definitions

- **Biosafety**

  Containment principles, technologies and practices that are implemented to prevent **unintentional** exposure of individuals and the environment (= *biocontainment*) to potentially hazardous biological agents

- **Biosecurity**

  Institutional and personal security measures designed to prevent loss, theft, misuse or **intentional** release of microorganisms, biological materials and research-related information

STORAGE OF PATHOGENS

Storage of live pathogens requires appropriate containment and security to avoid risks due to breakage or unauthorised use of material.

Storage facilities should be appropriately labelled to indicate the nature of the pathogens (e.g. their Group) and the contact information for the person(s) responsible for them.

A complete inventory of the pathogens in storage should be kept up to date and available.

Lessons to be Learned from Recent Biosafety Incidents in the United States

Shay Weiss PhD, Shmuel Yitzhaki PhD and Shmuel C. Shapira MD MPH

Department of Infectious Diseases, Israel Institute for Biological Research, Ness-Ziona, Israel

ABSTRACT: During recent months, the Centers for Disease Control and Prevention (CDC) announced the occurrence of three major biosafety incidents, raising serious concern about biosafety and biosecurity guideline implementation in the most prestigious agencies in the United States: the CDC, the National Institutes of Health (NIH) and the Federal Drug Administration (FDA). These lapses included: a) the mishandling of *Bacillus anthracis* spores potentially exposing dozens of employees to anthrax; b) the shipment of low pathogenic influenza virus unknowingly cross-contaminated with a highly pathogenic strain; and c) an inventory lapse of hundreds of samples of biological agents, including six vials of variola virus kept in a cold storage room for decades, unnoticed. In this review we present the published data on these events, report the CDC inquiry's main findings, and discuss the key lessons to be learnt to ensure safer scientific practice in biomedical and microbiological service and research laboratories.

Bacillus anthracis spores [2]. The incident occurred during
preliminary assessment as to whether MALDI-TOF mass-sp
trometry could provide faster detection. The BRAAT laborat
used, under BSL-3 containment conditions, a method for p
tein extraction that had been previously optimized for Bruc
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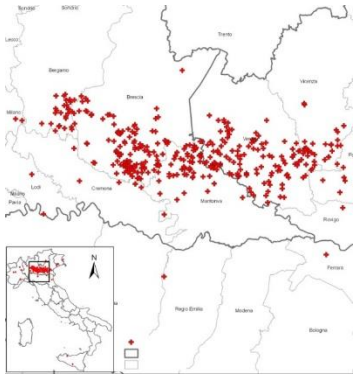
growth. This extraction method was never verified by the BRA
laboratory for its efficacy in inactivating the select bacterial ag
used in this case. In the BSL-3 BRAAT laboratory, each pro
extract sample had been divided into two aliquots: one filte

Cross-contamination of samples is a high risk factor in laboratories that use multiple microorganisms or species with different pathogenicity



Quality control

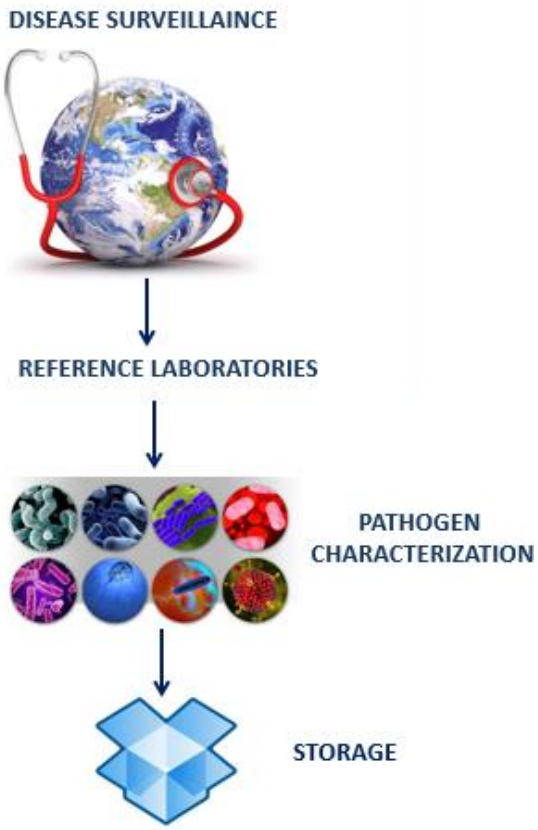
Storage

Establishment of a Veterinary Biobank for AI at Istituto Zooprofilattico Sperimentale delle Venezie

A biobank is a repository to store biological samples for their use in research. The scope of biobanks is to provide the scientific community access to scientific material and data.

Surveillance activities and scientific research at national and international levels have resulted in collection and storage of invaluable biological materials. Part of invaluable samples, collected during AI outbreaks, is now part of IZSVe Biobank.

The IZSVe Veterinary biobank assists the World Animal Health Organization in evaluating and adopting reference materials for animal disease diagnosis.



How to access

Biological materials present in the biobank can be searched on line at http://biowarehouse.net.

For further information: msbeato@izsvenezie.it

1st line **Conscientious and proficient laboratory staff** reduces the inherent risks that attend work with hazardous agents

2nd line **Safety equipment** remove or minimize the exposures to hazardous biological materials (Biological Safety Cabinets/BSCs)

3rd line **Suitable design and construction** of the facilities contributes to the laboratory workers‘ protection

Thank you for your attention!