VETBIONET

Veterinary Biocontained facility Network for excellence in animal infectiology research and experimentation

Deliverable D3.7

Guidelines for best practice in Post-Mortem facilities

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Table of contents:

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1. Summary

Objectives:

The risk potential for exposure to zoonotic pathogens is considered higher in a post-mortem room than in modern laboratory settings, with the associated risk to personnel working in there and release or spread to the environment. This increased risk is thought to be a result of the nature and size of the material being dealt with which cannot be done in primary containment, human factors of operatives when undertaking post-mortem examinations and procedures not suited for adventitious infectious hazards present in animal corpses. This is considered a priority to address by VetBioNet.

Through a workshop with project partners and concerned members of the GOHLD group and document review a systematic comparison of different processes and post-mortem facility designs in a bowtie biorisk control model was used to identify the risk control systems in use, both in terms of facility design and procedural practices. This information can be used to devise a self-assessment tool for facility operators, which can be used to prioritise continual improvement efforts and the design of new or refurbished facilities.

2. Introduction

Research animal facilities at containment level 2, 3, 4 (CL-2, CL-3 and CL-4) should have access to designated post-mortem facilities at the same containment level. Depending on the biological agents and animal species studied the risks of exposure to the biological agents under study and to adventitious biological agents that concurrently infect the study animals can pose a higher risk than during animal procedures and husbandry. For the adventitious biological agents, the containment measures depend on the harm benefit assessment balance of the hazard and the likelihood of infecting staff based on their prevalence, and biohazardous properties.

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3. Results

The workshop and review went through the various aspects of running a post-mortem. It reviewed the impact of the legal requirements applicable in the jurisdiction to the biorisk management system of PM room. These include

"Biological Agents Directive" (2000/54/EC)

The EU biological agents' directive is transposed into national legislation to protect workers from biological hazards as part of the health and safety at work legislative framework.

Animal Health Law

The EU animal health law of 2016, section 4 article 16, places and obligation on laboratories and other facilities handling animal pathogens, vaccine agents and biological materials to apply biosecurity, biosafety and biocontainment measures with the purpose to prevent the exposure of animals outside the facility

OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals

All EU member states have agreed to implement the chapter 1.1.4 Biosafety and Biosecurity: Standard for Managing Biological Risk in the Veterinary Laboratory and Animal Facility. This fundamentally requires a risk assessment process, which identifies risks and establishes controls for all risk paths leading to environmental release or operator exposure for zoonotic agents.

Animal By-Products Regulations

Animal carcasses, tissues and effluents from infectious disease studies are by definition category 1 animal by-products and have to be inactivated and disposed by approved processes

Biocidal Products Regulations 528/2012

Under Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products only approved disinfectants can be marketed and used in the EU. Disinfectants are restricted to applications in line with their Product Type approval.

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Agreement on Dangerous Goods by Road (ADR)

Animal carcasses suspected to be infected with human or animal pathogens in categories A and B have to be transported under the provisions of the ADR

Urban Waste Water Treatment Directive 91/271/EEC

This directive prescribes the treatment of waste water to protect the health and safety of staff working in sewers and municipal water treatment plants. Applied to necropsy facilities this is understood to require the inactivation of biological hazards and potential chemical hazards.

Barrier based Risk Control Strategy using a Bowtie Risk Model.

The report also covers an infection risk control strategy for the operators and the releasing of zoonotic and epizootic pathogens from the facility.

The approach used in the exercise to establish to a balanced risk management strategy was the application of the Biorisk bowtie model, which lends itself for biological risks. All biological risks have a *biohazard source* and develop along typical *risk path* to a *loss of control event* before escalating along multiple alternative risk paths resulting in the adverse consequences. The typical loss of control events (LOCE) are the release outside the containment barriers or the infectious exposure of one or more operators. The different consequences in terms of health damage, reputational damage, financial damage and economic damage determine the rigour and number of protection layers that are applied to prevent the *loss of control event.*

The facility risk assessment reviews all processes in the facility for their potential to result in the occurrence of the LOCEs and identifies complementing protection layers that reduce the likelihood of a LOCE to a negligible level. Where this is not sufficient mitigation controls are determined that slow down or mitigate the consequences from loss of control events

Process Mapping in a Veterinary Necropsy Facilities

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To undertake this risk assessment, and subsequent planning the biological risk control systems, an analysis of all processes and how they are connected was undertaken by the group. This was based on the process map for a veterinary necropsy facility where each process was looked at for specific typical risk points that characterise the risk paths, which lead to a loss of control. However, the risk point tables drawn up by the group and reported should only intended as a starting point for a facility risk assessment, due to the potential for variation between facility design and use.

- Process reviewed included
- Receiving carcasses
- Euthanasia for Animals
- Carcass movement and storage
- Post-mortem procedures, including handling blood and body fluids, cutting bones and taking of samples
- Processing of samples including culturing and other techniques for detection of biological agents and cryostat histology
- Management of waste flows including effluents from the post-mortem facility

These design of facilities was looked at where these various processes were to be undertaken, the risk paths and the corresponding biorisk control systems that should be in place to mitigate these risks.

These included

- Controls for preventing liquid release, including effluent drains and collections system, flood prevention systems, modalities for the decontamination of necropsy effluents.
- Controls for air-borne release and exposure, including containing aerosol generating processes, engineering controls to prevent both operator exposure and release as well as release via drainage vents, and effluent collection and decontamination systems
- Controlling release and exposure by fomites through various operating and sterilisation processes
- Facility decontamination and decommissioning
- Disposal of carcasses, animal tissues including blood, other clinical waste and chemicals, including movement off site

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- Movement of operators including their use of personal protective equipment (including respiratory protective equipment)

The full report is placed in the addendum and includes links to appropriate references, legislation and information.

4. Conclusion

The output of the workshop and review covered both the legal aspects of operating a Post-Mortem room and used bowtie biorisk control model was used to identify the risk control systems in use, both in terms of facility design and procedural practices. This information can be used to devise a self-assessment tool for facility operators, which can be used to prioritise continual improvement efforts and the design of new or refurbished facilities.

The report was placed on the VetBioNet project shared workspace and will be shortly either be placed on the VetBioNet area of the International Veterinary Biosafety Working group (IVBW) site (which is linked from the VetBioNet website) or published in peer review open access journal and linked on the IVBW site.

5. Addendum – Best practice in Post-Mortem Facilities

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Best Practice Biosafety in Post-Mortem Facilities

Guidance on biorisk management in veterinary post-mortem facilities

Deliverable 3.7

Vetbionet

1 Introduction

The risk potential for exposure to zoonotic pathogens in pathology settings in inarguably higher than in modern laboratory settings. This is a result of poor infrastructure, human factors, and procedures not suited for adventitious infectious hazards present in the cadavers [1–3]. Investigative pathology is crucial for understanding the pathophysiology of disease conditions and cannot be replaced by clinical laboratory data or biopsies alone [4,5]. In the course of the risk assessment for intentional work with biological agents in animals, the same safety principles apply during necropsy as in a microbiological laboratory. However, the biological agents' directive (2000/54) excludes post-mortem facilities from the scope, so the design and practices are not prescribed at European level. The comparison of practices and facility designs adopted by Vetbionet partners identified common practices and differences, which are the basis of this guidance. Good necrospy facilities are expensive and the available guidance is limited and not broadly adopted by designers. Here we attempt to capture current good practices with a focus on biorisk management aspects. The general design considerations for veterinary necropsy facilities have been described Citino [6]. For a necropsy suite serving a high containment facility biorisk control measures require even more focus.

The risk tolerance for the incidental necropsies on carcasses infected with zoonotic agents differs to mortems carried out on animals intentionally infected with high consequence pathogens. Upon careful assessment the spectrum of zoonotic agents present in field necropsies is broad and given the availability of good engineering control there is no good reason, why a pathologist should have to accept a much higher risk of infection than other professions.

In the laboratory samples of unknown infectious status are handled in Containment Level 2 with universal precautions. A similar set of universal precautions is adopted in veterinary necropsy facilities to control the most obvious routes of Adventitious infections with zoonotic pathogens. Additional measures should be adopted for the intentional work on carcasses infected with zoonotic and high consequence animal pathogens.

1.1 Scope

Research animal facilities at containment level 2, 3, 4 (CL-2, CL-3 and CL-4) should have access to designated post-mortem facilities at the same containment level. Depending on the biological agents and animal species studied the risks of exposure to the biological agents under study and to adventitious biological agents that concurrently infect the study animals can pose a higher risk than during animal procedures and husbandry. For the adventitious biological agents, the containment measures depend on the harm benefit assessment balance of the hazard and the likelihood of infecting staff based on their prevalence, and biohazardous properties.

A recently constructed post-mortem facility for human corpses infected with high consequence pathogens suspected to be infected with haemorrhagic fever viruses encountered very similar challenges, highlighting that the human pathology sector also needs to look for new ways to improve the safety of post-mortem facilities.

1.2 History

Occupational exposure associated infections in pathology facilities are a recognised challenge. Due to the small number of facilities and no central reporting system such incidents do not regularly get published. Health statistics for pathology workers are not available to leverage the investment into contemporary biosafety equipment. The infection risk of tuberculosis associated with post-mortems was reported as 2-9 times higher than the general population in 1957 [7] and again 5 times higher in a survey in 1976 [8]. Despite this specific risk, the levels of viable bacteria in air samples from post-mortem rooms suggest that the majority of viable colony forming units are derived from the staff and not from the corpses, with overall counts that do not exceed surgical suites [9].

Some of theow are a few examples of infections associated with post-mortem or abattoir work:

1967: Haemorrhagic Fever Disease was described in laboratory workers and animal handlers in research facilities in Marburg, Frankfurt and Belgrade, which led to the discovery of Marburg Virus [10].

1992: A Q Fever outbreak with approx. 80 confirmed infections was associated with a veterinary clinic and post-mortem facility [11]. The scale of outbreak was attributed to the ventilation system, which was believed to have spread the infection into administrative areas of the pathology building. Other risk paths related to the ventilation in the room, the use of PPE, the barrier procedures and the management of PPE (personal communication UMD).

1996. At an ostrich abattoir 17 workers contracted Crimean Congo Haemorrhagic Fever [12]. The infected workers decreased along the processing line. Maintaining the ostriches 14 days prior to slaughter in a tick free environment was enough to prevent the slaughter of viraemic birds.

2011: Exposure of four workers with *Mycobacterium tuberculosis* during the necropsy of a dog submitted with the anamnesis of a brain tumour. Fortunately, the M tuberculosis strain was susceptible to antibiotics, so the infection could be treated readily [3].

1.3 Cost Benefit Assessment

- People outside the post-mortem facility should be at no increased risk of infection due to the proximity to the facility.
- For the facility workers the risk of exposure will inevitably be higher, but should be as low as reasonably practicable (ALARP) [13]. A more quantitative approach to control

the risks and determine what the permissible cost for ALARP controls or for the *best available technologies not entailing excessive cost* (BATNEEC) are lacking and suffer from an overall small population of veterinary pathologists.

The risk tolerance is ultimately a matter Whether cost is excessive is ultimately a socioeconomic cost benefit calculation, as an orientation the cost should exceed the damage by a factor 10 before it can be considered excessive. Different countries apply different cost to lost time injuries (infections), but under normal conditions the risk for operators should not be more than 10x higher than for the general population. This should include single point failures of risk controls.

Since the risk is not clearly quantifiable based on reporting of pathology related morbidity, cost benefit calculations can be contentious.

2 Regulatory requirements

To ensures compliance with the legal requirements applicable in the jurisdiction, the biorisk management system has to be developed considering these requirements. The EU directives are transposed into national law, which may differ slightly between Member States, the EU regulations are directly applicable in all Eu member states but may be interpretable.

2.1 "Biological Agents Directive" (2000/54/EC)

The EU biological agents directive [14] is transposed into national legislation to protect workers from biological hazards as part of the health and safety at work legislative framework. Requirements Post-mortem facilities are not specified and the general requirements for laboratories and animal facilities have to be applied.

2.2 Genetically Modified Organism Contained Use Directive (2009/41/EC)

Implemented through member state legislation the EU genetically modified organism contained use directive [15] is providing biosafety controls for genetically modified microorganisms. These controls aim further than the biological agents directive in the expectation that there is no release rather than no consequential release.

2.3 Animal Health Law

The EU animal health law of 2016 [16], section 4 article 16[1], places and obligation on laboratories and other facilities handling animal pathogens, vaccine agents and biological

[1] Section 4 Article 16: Obligations of laboratories, facilities and others handling disease agents, vaccines and other biological products

1. Laboratories, facilities and other natural or legal persons handling disease agents for the purpose of research, education, diagnosis or the production of vaccines and other biological products shall, whilst taking into account any relevant international standards:

(a) take appropriate biosecurity, biosafety and bio–containment measures to prevent the escape of the disease agents and their subsequent contact with animals outside the laboratory or other facility handling disease agents for those purposes;

materials to apply biosecurity, biosafety and biocontainment measures with the purpose to prevent the exposure of animals outside the facility. Some more detailed requirements are found in the directives for specific epizootic animal diseases or in the Diagnostic Manuals implemented by decision of the European Commission (see table) These include high level biosafety requirements for avian influenza, African swine fever, classical swine fever, foot and mouth disease, and Newcastle Disease. In some Member States specific legislation for high consequence animal diseases is in place to regulate work on epizootic animal diseases, e.g. the Specified Animal Pathogens Order in the United Kingdom.

(b) ensure that the movement of disease agents, vaccines and other biological products between laboratories or other facilities does not give rise to a risk of the spread of listed and emerging diseases

Disease	Directive controlling the Disease	Facility biosafety requirements
Classical Swine Fever	Council Directive 2001/89/EC[17]	Commission Decision 2002/106/EC [18] - limited key requirements for animal facilities, e.g. post-mortems must be performed in the bio-safe area.
Foot and Mouth Disease	Council Directive 90/423/EEC[19]	Minimum Biorisk Management Standards for Laboratories working with Moot-And-Mouth Disease Virus [20]- very detailed and prescriptive, but little specifics on post-mortem facilities
African Swine Fever	Council Directive 2002/60/EC [21]	Commission Decision 2003/422/EC Chapter VIII [22] -very limited key requirements for animal facilities, e.g. post-mortems must be performed in the bio-safe area.
Avian Influenza	Council Directive 2005/94/EC [23]	COMMISSION DECISION 2006/437/EC[24]
Newcastle Disease	Council Directive 92/66/EEC [25]	No specifics other than requirement for post-mortem facilities at diagnostic laboratories

2.4 OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals

All EU member states have agreed to implement the chapter 1.1.4 Biosafety and Biosecurity: Standard for Managing Biological Risk in the Veterinary Laboratory and Animal Facility [26]. This fundamentally requires a risk assessment process, which identifies risks and establishes controls for all risk paths leading to environmental release or operator exposure for zoonotic agents.

2.5 Animal By-Products Regulations

Animal carcasses, tissues and effluents from infectious disease studies are by definition category 1 animal by-products [27] and have to be inactivated and disposed by approved processes [28]. In Annex IV of regulation 142/2011 [28] the requirements for effluent are specified and prohibit the discharge of blood and animal by-products by the effluent route and specify a pre-treatment of effluents from animal by-product facilities. The intent of 2000/54 and 41/2009 is that animal carcasses are inactivated before they leave the controls of the biocontainment facility.

2.6 Biocidal Products Regulations 528/2012

Under Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products [29] only approved disinfectants can be marketed and used in the EU. Disinfectants are restricted to applications in line with their Product Type approval.

2.7 Agreement on Dangerous Goods by Road (ADR)

Animal carcasses suspected to be infected with human or animal pathogens in categories A and B have to be transported under the provisions of the ADR[30]. Depending on the suspected or confirmed pathogen the shipments have to be classified as UN2814 animals only, UN2900 animals only, and UN3373. After thermal inactivation the remains are transported as animal by-products, but no longer classify as hazardous good and do not have to be classified under ADR. Transport of animal carcasses infected with high consequence disease is appropriate for the journey from the field to the post-mortem facility. Animals infected with biological agents as part of research studies should not be removed from the biocontainment facility without inactivation. For this intentional work appropriate inactivation equipment must be provided in research biocontainment facilities.

2.8 Urban Waste Water Treatment Directive 91/271/EEC [31]

This directive prescribes the treatment of waste water to protect the health and safety of staff working in sewers and municipal water treatment plants. Applied to necropsy facilities this is understood to require the inactivation of biological hazards and potential chemical hazards. The transposition of this directive may differ between EU member states.

3 Risk Assessment

3.1 What are the risks

The primary risks considered here are infection risks for the operators and the risk of releasing zoonotic and epizootic pathogens from the facility. The agents studied are immediately on the mind of all concerned. Zoonotic agents that are endemic in the country are on the mind of the diagnostic pathologist, but sometimes neglected, when they are present as adventitious infections in research animals. Due to the change in environment latent infections can resurface and pose an additional infection risk in animals

The primary focus is to avoid operator exposure to zoonotic disease agents. In routine submissions to a post-mortem facility the likelihood of exotic disease agents is low, but in biocontainment facilities it is the daily routine. However, adventitious zoonotic agents can in addition be present in outbred livestock species that were not purpose bred under SPF conditions. If epizootic diseases are suspected post-mortems are avoided to limit the transport and dispersion of the disease.

The challenging question is for which zoonotic disease agents should one always prepare for as adventitious infections not mentioned in the Anamnesis or not screened for when sourcing study animals: *Coxiella burnetii, Mycobacterium bovis*, *Campylobacter spp.*, *Echinococcus spp.*, *Francisella tularensis,* Hantavirus, Hepatitis E virus, etc

In addition, other hazards need to be considered and assessed, and engineered out as far possible but are not addressed in detail here: e.g. manual handling of heavy carcasses (lifting equipment), chemical hazards from formaldehyde and disinfectants, chemical hazards from gaseous decontamination, ergonomic working postures (height adjustable tables and safety cabinets), slip and trip hazards, electrical hazards in wet rooms.

3.2 Barrier based Risk Control Strategy using a Bowtie Risk Model.

One approach to drive to a balanced risk management strategy is the application of the Biorisk bowtie model, which lends itself for biological risks. All biological risks have a *biohazard source* and develop along typical *risk path* to a *loss of control event* before escalating along multiple alternative risk paths resulting in the adverse consequences. The typical loss of control events (LOCE) are the release outside the containment barriers or the infectious exposure of one or more operators. The different consequences in terms of health damage, reputational damage, financial damage and economic damage determine the rigour and number of protection layers that are applied to prevent the *loss of control event.*

The facility risk assessment reviews all processes in the facility for their potential to result in the occurrence of the LOCEs and identifies complementing protection layers that reduce the likelihood of a LOCE to a negligible level. Where this is not sufficient mitigation controls are determined that slow down or mitigate the consequences from loss of control events.

4 Process Mapping in a Veterinary Necropsy Facilities

The first step in conducting the risk assessment and planning the biological risk control systems is an analysis of all processes and how they are connected. Based on the process map, as each process has specific typical risk points that characterise the risk paths, which lead to a loss of control. The typical risk point tables at the end of process description cannot be comprehensive and are only intended as a starting point for a facility risk assessment.

4.1 Receiving Carcasses

Diseased animals or carcasses are transported to the necropsy facility. For experimental studies the necropsy facility should always be part of the biological containment animal facility. If carcasses infected with infectious diseases are transported to the post-mortem

facility area for unloading have to provide appropriate facilities for decontaminating and cleaning the vehicles and any surfaces that may get soiled during unloading. When departing the delivery vehicles should be sanitised and should not pose a risk to other animal holding after leaving the PM facility. The vehicles need to be cleaned and disinfected under a roof to separate the run- off water from rainwater and directed to the waste water treatment. An interceptor tank, which permits directing the water from spills or deliveries of concern to a biowaste decontamination system is advisable if zoonotic or epizootic diseases are taken into consideration. Cleaning should minimize aerosol formation until all surfaces are disinfected.

Typical Risk Points	Controls
Uncontrolled movement of people across the containment boundary	Airlock for access to a biocontainment PM facility
Contamination of the delivery trucks from within the facility	Decontamination process for the airlock after every use.
Vehicles delivering animals leave the premise without decontamination	Covered area for cleaning and disinfection of delivery vehicles
People delivering carcasses or animals get exposed to biological agents from the facility	Clear segregation between clean areas and potentially contaminated areas. Clear standards of packaging carcasses for delivery.

4.2 Euthanasia of Animals

In research facilities working with livestock species, a dedicated area for euthanasia is required. To address animal welfare euthanasia must not occur in occupied animal holding rooms and in active necropsy areas. The species specific requirements are defined at EU level [32] and by the OIE [33] The areas needs to enable the transfer of the carcasses to the necropsy room. In containment facilities, the animal holding area should be directly connected to the euthanasia and necropsy areas, permitting movement of animals within one environmental biocontainment boundary. The euthanasia area should be located and designed to minimize the stress for the animals. They should not be exposed to the smells or sight of blood or the necropsy. A door separating the two areas and a directional airflow from the euthanasia to the necropsy area address this. To ensure the safety of the workers it is important that there is enough space to handle the animals and retreat to a safe area if animals panic. Equipment to restrain large animals needs to suit the procedures in each facility. Lifting equipment to move the carcasses safely to the necropsy area may also be used to exsanguinate the animals after stunning. If animals are stunned and bled out the lifting equipment has to lift the stunned animals with appropriate speed to complete the process within the permitted maximum (Stun-stick interval). From a biosafety perspective the equipment has to be selected so it can be decontaminated and all materials are compatible

with the disinfectants in use. While stainless steel equipment may be the first choice, galvanised steel can be considered as long as the blow holes are permanently sealed and all burs that may rip gloves have been polished off to provide smooth surfaces with no sharp edges.

Typical Risk Points	Controls
Aerosol generation during handling, stunning and exsanguination	Ventilation and air filtration, directional airflow, PPE and where appropriate RPE
	Consider euthanasia by injection if compatible with the scientific objectives
Blood flowing into the floor gully	Means to collect blood and prevent it from discharge to floor gullies.
Reverse contamination of the animal facility from the shared necropsy facility	Directional airflow pressure cascade protecting clean spaces and spaces of lower contamination. Airlocks and or airtight doors to separate zones of different contamination

4.3 Storing Carcasses

Carcass storage areas should meet the same hygiene requirements as the PM room. The air change rate will be low to avoid excessive condensation, however when accessed by personnel, a good air change rate should be provided. Ideally, all carcasses are packaged in containers or bags while in the cold store, fridge or freezer so that the cold store does not routinely get soiled. To maintain a stable temperature in the cold room full height doors should only be opened to move carcasses on overhead rails. A normal height door for operators moving trolleys or bins should also be provided.

Typical Risk Points	Controls
Contamination of the cold room the room, fridge or deep freezer by open stored carcasses, with limited ability to clean while in use.	Primary packaging of carcasses
Management of contaminated containers and bins used for carcass and waste storage	Decontamination and cleaning process for equipment that minimises operator exposure and provides a validated decontamination

4.4 Internal Movement of carcasses

Carcasses of large animal species are best transported on overhead rails. These can be designed to extend through doorways. In order to handle carcasses of large animal species the rooms have to have a height of 4-5 metres (1m for the rail and crane, 3 metres for the stretched carcass and ropes and 1m to lift the carcasses onto a table). Overhead rails are the safest and most efficient way to transport large carcasses but come with the biosafety concern that they generate surfaces not accessible for routine cleaning and disinfection. Some facilities have provided only one hoist to lift carcasses up for exsanguination, others have completely avoided overhead rails and use carts to move the carcasses if required with motorised machinery. Some facilities use mobile cranes for engine blocks to lift carcasses on and off necropsy tables instead of overhead rails. Ceiling mounted rails are still the preferred technology in facilities handling cattle and adult pigs. Strategies to manage overhead rails may include displacement ventilation, i.e. high level supplies and low level returns, UV-C irradiation of the room head space, and scissor lifts to access ceiling mounted equipment for routine cleaning and maintenance.

Typical Risk Points	Controls
Splashing and contamination of the floor with discharges from transported animals.	Consider plastic shrouds that capture discharges from hanging carcasses
Transport using overhead rails requires very high rooms (4,5 to 5m), which require special considerations for cleaning	Displacement ventilation
	UV-C head space irradiation
	Scissorlift for decontamination at high level.
Ability to decontaminate equipment, e.g. mobile cranes, scissorlift, hoist, mobile containers and trolleys for transporting carcasses	Waterproof design of equipment.
	Decontamination and cleaning process for equipment that minimises operator exposure and provides a validated decontamination

4.5 Post-mortem Procedures

The dissection itself generates bioaerosols and droplets, although the quantity is very much dependent on the techniques and the extent of the necropsy. Aerosols are best contained at source. For small laboratory animals it is feasible to conduct the entire post-mortem in a class I biological safety cabinet and this is the recommended approach for all small carcasses,

where zoonotic disease agents are studied or suspected, e.g. bats or wild rodents. For larger animals that cannot be handled in a biosafety cabinet a downdraft table would be the next best option. Some facilities are using downdraft tables but the feedback of pathologists from multiple facilities suggests that the operators prefer the use of protective suites with respiratory protection to avoid exposure to aerosols. This is Some facilities use down draft tables but mainly for formaldehyde extraction. Downdraft tables for necropsy can offer protection from aerosols but most models seem to struggle handling water, are very difficult to decontaminate afterwards.

The different facilities handling zoonotic agents varied in their approach from working carefully to avoid the generation or aerosols, using RPE, using suits with RPE, using Class I biological safety cabinets, or downdraft tables.

Typical Risk Points	Controls
Dissecting carcasses	Dissection of carcasses infected with zoonotic pathogens should be limited to the extent required for the scientific goals.
Infectious splashes and aerosol generation	Class I biosafety cabinets
	Downdraft tables, local exhaust ventilation
	Laminar air supply above the work area
	Face shields, Respiratory protective equipment
Contamination of the PPE and floor with infectious body fluids	Post-mortem tables with a liquid collection system to limit the contamination of the floor and prevent blood from draining into the floor drains.
	Selection of PPE and a matching process to clean and disinfect PPE before degowning

4.6 Special Procedures: Handling Blood and body fluids

Blood must be captured and disposed of as animal by-product and is not permitted in the facility waste water [28]. The blood can be collected in a bed of coarse wood wool placed in the bottom of a plastic bag or bucket to minimise splashing and accelerate coagulation. Wood wool creates less dust and is more easily managed than sawdust or wood shavings.

Typical Risk Points	Controls
Body fluids and bloods draining to the	Design tables with fluid collection systems

floor drain	
	Consider a blood collection mechanism if exsanguination is required.
	Drain plug for floor drains during exsanguination.
Contamination of HEPA filters and the interior components of downdraft tables	Downdraft table design

4.7 Special Procedures: cutting bones

Pathologists cut bones to examine the bone marrow, the spinal cord or the brain. Depending on the task they use oscillating saws and band saws preferentially. Both tools are notorious for generating very high amounts of aerosols [34–36]. Hand saws combine significantly fewer aerosols with other practical draw backs [36]. Formalin Fixed samples for histology, Tissue Trimming

Formaldehyde vapours from cutting formalin fixed tissue blocks should be extracted on downdraft cutting workstation. containers with fresh formalin and containers holding fixed tissues should be extracted in a vented chemical cupboard. Both activities are not infectious and should be separated from the necropsy area.

Typical Risk Points	Controls
Insufficiently fixed tissues are handled, which are still infectious	The laboratory area for handling tissues from the necropsy should meet the same containment as the PM facility
	Strict procedures for sizing tissue blocks, ratio of tissue to formalin and the fixation time to ensure at the point of trimming all tissue samples are fully fixed
Safe Disposal potentially infectious formalin	Formalin is inactivating all high hazard biological agents if sufficient time and concentration is maintained.

4.8 Sampling for microbiology and toxicology

Samples for microbiology and toxicology may have to be shipped to specialist laboratories. These can only receive the samples if either they have the appropriate biosafety. If the detection is based on PCR the samples can be stored in a microbicidal denaturing buffer or the nucleic acid can be extracted before shipping. If zoonotic biological agents a

4.9 Cryostat histology

Sectioning frozen tissues is one of the exposure prone activities in the laboratory. As the Cryostats are not designed to provide protective inward directed airflow, they rely almost entirely on good microbiological techniques to prevent spread of contamination. Some models are equipped with UV-C light to improve pathogen inactivation and some have contained systems for collecting condensate and for extracting waste at source. They need regular decontamination and good training to minimise the infection risks. The blade should only be changed with steel msh gloves. They need to be disinfected after handling infectious samples.

Typical Risk Points	Controls
Infectious aerosol release	Good technique and RPE. Local exhaust ventilation at the front opening.
Cryostat cannot be decontaminated like other equipment in the necropsy suite	The cryostat should be designed so it can be easily cleaned and disinfected. It should be sited in a laboratory that provides secondary containment.
Safe Disposal potentially infectious formalin	Formalin is inactivating all high hazard biological agents if enough time and concentration is maintained.

4.10 Photography

Photographic equipment is not easily disinfected, so it best located in a supporting laboratory, which does not get contaminated with infectious aerosols.

Typical Risk Points	Controls
Contamination of photographic equipment not designed for liquid cleaning and disinfection	Directional airflow protecting the photography room from airborne contaminants in the necropsy area
Contamination of the photography room by necropsy operators	Procedure for decontaminating before entry to the photography room

4.11 Biological samples for the detection and culture of biological agents

In order to isolate biological agents, tissue samples have to be submitted to diagnostic laboratories, where these samples will be processed in closed systems or in biological safety cabinets. All containers removed from the PM suite have to be surface disinfected to remove them from the PM suite and transported in a secondary leak proof container to the laboratory. The disinfected primary containers can be passed through the containment barrier in pass boxes, gaseous decontamination chambers or through dunk tanks. It is

recommended that they are transferred out separately from the operator exit procedure to avoid human error.

Typical Risk Points	Controls
Spills from the primary containers	Protect primary containers in a leakproof secondary container.
Contamination of the receiving with contamination from leaky primary containers	Secondary containers is only opened inside biosafety cabinet. It has to be sized accordingly

4.12 Management of effluents from the post-mortem facility

During the necropsy biological agents are released that are normally contained in the carcass. The intentional process of dissecting carcasses for diagnosis and research is thus generating a risk in effluents. The inactivation of biological hazards in the effluents is good practice to demonstrate control. In most cases an effluent decontamination system is required, unless a post-mortem facility is only handling SPF animals. Intentional work with human pathogens/zoonotic pathogens requires validation inactivation under EU directives 2000/54 [14] and under the Urban Waste water treatment directive (1991/271/EEC), treatment of industrial effluents is required to protect the health and safety of people working on sewers and in wastewater treatment plants [31]. For genetically modified organisms inactivation is required under directive 2009/41 [15] and under the EU Animal Health Law 2016/429 [16] an obligation is placed on laboratories and facilities "to take appropriate biosecurity, biosafety and biocontainment measures to prevent the escape of the disease agents". Finally, the Animal By-Product Regulations [27,28] require effluent pre-treatment for facilities handling animal by-products. Neither blood, nor particles above 6mm diameter can be released to the sewer.

Due to the overlapping legal requirements the scope and requirements for effluent decontamination system may require clarification with one or several competent authorities.

Which effluents should be included in the treatment?

Effluent source	Comments
Necropsy room floor drains, and utility sinks	At CL-2 and above the effluent from the activity should be treated by a validated means.
Hand wash sinks at the exit from the facility	At CL-2 this effluent does not have to be treated, assuming that hands do not get contaminated, at CL-3 and CL-4 it is required
Boot washing drains	Where dedicated boot wash stations are installed

	they are part of the contaminated effluent stream.
Run-off water from the animal unloading and vehicle cleaning and disinfection activity	This area should be covered so rain water does not enter the drain. Dependent on assessment this area may be directed to drains based on risk assessment
Autoclave condensate	Decontamination autoclaves should be designed with reliable controls for condensate retention and sterilisation, Connecting the sterilised drain to the contained drains
Laundry Machine discharge	Depending on the machine and wash cycle the early wash stages may not be decontaminated
Parts washer	Parts washers may discharge contaminated effluent from prewash cycles.

Typical Risk Points	Example Controls/ Comments
Confusion as to which drain points are treated	Clear, and intuitive principles, e.g. all drains inside the biocontainment barrier are treated
Animal By-products entering the drains	Combination of strainers with <6mm mesh size and procedures to capture blood and body fluids
Damage to drain pipes resulting in environmental leaks	Design drains to be accessible for inspection, e.g. basement; Drainage design providing secondary containment, ability to verify drain integrity at any time, drain integrity alarms
Corrosion of pipes resulting in leaks	Ensure drain material and chemicals/disinfectants used in the facility are compatible.
Technical staff operating and maintaining drains and effluent decontamination system are exposed to biological agents	The effluent decontamination system should be a closed process separating the people from the infectious material. The drainage system must be designed for decontamination, e.g. by heat or flooding

	with caustic soda (NaOH). The effluent collection tank has to permit steam decontamination and cleaning in place with NaOH at elevated temperatures (70-90°C). Procedures to decontaminate drains and all components of the EDS for maintenance.
Drain content both liquid or air contaminating drain points in other rooms	Processes to maintain the air seal on trap and detect blockages in the drains early.
Effluent decontamination process is not effective for the actual effluent composition	Process validation with worst case effluent composition
Failure of the effluent decontamination process permitting the release of untreated /infectious waste water	Careful system design, using experienced designers. Structured system safety qualification including PHA at design stage and staged verification and commissioning of the entire system.
Flooding of the effluent collection system due to excessive water usage, or leaks from water taps	Metered Water supply with alarmed usage profiles and interlocks to prevent excessive water usage
Blocked drains cause a flood of the facility and liquid release to the outside or adjacent facility areas	Floor drain strainers have to be sized to minimize this risk of blocking within one shift. Design the floor drain gullies to enable easy and safe decontamination and cleaning.
	The facility floors have to fall towards contained drains.
	Floor to wall transitions have to be leaktight and easily cleanable.
	Design the facility water from cleaning the floors or floods is retained in the facility by bunds and ramps where for equipment or containers on wheels is required
Overfilling effluent collection system	The collection tank should be operated with sufficient reserve (head space) to accommodate unusual usage patterns. To benefit from this there should be alarms communicating to the operators when to

	stop water usage leaving enough reserve for the exit decontamination process.
Flooding of the Effluent decontamination system (EDS) suite by leakage	The suite housing the EDS should be designed as a cleanable space. The EDS should be placed inside a bund that can retain minimum 110% of the largest connected tank system.

4.13 Waste Flows

The waste flows from pathology facilities differ only in a few points from other biocontainment facilities, needing special consideration, specifically laundry, personal protective equipment, tools, carcasses, formalin treated material and effluents. As far as practicable all waste should be autoclaved before further cleaning takes place. Laundry may be washed in the facility using high temperature programmes.

5 PM Facility Functional Areas.

The facility footprint needs to include the main necropsy room(s) and supporting rooms to accommodate all functions and processes required for the facility: As far as reasonably possible processes involving biological agents are most safety and efficiently handled in primary containment, however processes involving necropsies on livestock species that cannot be safely handled in biosafety cabinets as primary containment are carried out in the post-mortem room and operators are relying on good microbiological techniques, room ventilation and their personal protective equipment for protection (primary containment rooms). The room barriers also have to prevent the environmental release of biological agents handled in this way. When analysing the different functions and processes it is best to minimise the rooms that are primary containment. Secondary containment rooms are generally easier to manage and the requirements on the hygienic design for cleanability of equipment are not as stringent.

Room	Functions	biorisk[2]	Biosafety Considerations

[2] + Primary containment room: open handling of infectious material, protection by room ventilation, PPE/RPE

(+) Secondary containment room: infectious material handled in open primary containment for aerosol control, some fully enclosed other primary containment systems with dynamic protective airflow (e.g. Biological safety cabinets class I and II. Room contamination possible from procedural failures, spill incidents, and equipment failures.

((+)) Infectious material in validated closed system for primary containment, e.g. Effluent decontamination system, carcass disposal system, autoclave, potential room contamination during equipment breakdowns.

0 Uncontained areas outside of the biocontainment zone.

Room	Functions	biorisk[2]	Biosafety Considerations
Main Necropsy Room	Necropsy table(s) Primary containment Band Saw Primary containment oscillating saw Transport of large animals on overhead rails	+	Primary containment room. Should be the pressure sink
Euthanasia Room	Euthanasia of potentially infected animals. Collection of blood if animals are exsanguinated.	+	If the animal facility and post-mortem facility are handling a different pathogen portfolio at any time, zoonotic versus non-zoonotic and require different level of PPE, a separating airlock is advisable to better control the separation of the two facilities.
Carcass Delivery Airlock	for bringing carcasses in without breaching the containment to the outside, connected ot overhead rails	(+)/+	Interlocked doors, air purge and surface decontamination before exterior doors are released
Cold store	Storage of Carcasses before necropsy and before disposal Waste storage before disposal	(+)/+	Should be maintained as clean as possible, best if carcasses can be packaged.
2nd necropsy room with class I biosafety cabinet	Necropsies in primary containment Necropsies on small animals, including zoonotic diseases	(+)	Biological safety cabinet class I can also be used for detailed examination of organs. Avoid Class II cabinets as they are typically harder to clean.
Supporting	Photography,	(+)	All activities that do not require active open handling

Room	Functions	biorisk[2]	Biosafety Considerations
Laboratory	Class I or II biosafety cabinet Preparation of tissue samples for transfer to other laboratory areas, Bacteriology, Microscope for Quick Diff Cryostat Formalin container storage cabinet and tissue trimming downdraft table		of biological material should be separated away from the primary containment post-mortem room into secondary containment support laboratory. Pass through cabinets enable the movement of samples from the primary containment room to the support laboratory after surface disinfection.
Carcass Disposal	Loading carcass material into the carcass Disposal system	+	Loading mechanism should prevent and/or contain aerosols.
Carcass Disposal Equipment	Separated by a biocontainment barrier	((+))	The Carcass disposal system, regardless of the technology needs to be located in a room that can contain a spill and can be easily decontaminated. The primary containment barrier is critical, to ensure for normal operation the room is clean and unloading decontaminated carcass remains does not risk re-contaminating them.
Autoclave loading	Decontamination of disposable clinical waste, and reusable tools and equipment	(+)	The autoclave should not be loaded from a primary containment room.
Autoclave unloading	Double ended autoclaves are standard for	((+))/0	Autoclave integration into the barrier; condensate retention, hydrophobic sterile filters in the vacuum

Room	Functions	biorisk[2]	Biosafety Considerations
			line;
Technical Area for HVAC,	Outside of the biocontainment areas		Location of exhaust air prefilters, location of HEPA filters. Decontamination of filters, facilities for filter in situ testing. How is potentially contaminated ductwork decontaminated?
Effluent decontamination Suite	Raw effluent storage vessels, Effluent kill system, can be combined with carcass disposal unit. Personnel change barrier.	((+))	This room is normally a clean space secondary containment but can get contaminated when components break, e.g. pumps, valves, or system break downs require reactive maintenance without decontamination of the system or component.
PPE decontamination	C&D of apron, rubber boots, Removal of any contaminated PPE	+	
Degowning Room	Step-over separating clean and potentially contaminated side of the room, Handwash station	+	
Decontamination shower airlock	A barrier shower to decontaminate staff if PPE was breached/contaminated, or as a routine exit procedure for high risk zoonotic agents.	+	Laminar displacement ventilation system so aerosols formed during shower are extracted at low level, nominal air change rate >300 air changes per hour (>20cm/s linear velocity)
Clean PPE storage		0	
Laundry for scrubs	Used but not visibly	(+)	The soluble bags dissolve at

Room	Functions	biorisk[2]	Biosafety Considerations
	soiled scrubs are processed in sealed soluble laundry bags. Soiled scrubs are first autoclaved.		>60°C. Decontamination of the biological agents relies on the temperature and detergents.
Locker rooms	Street clothing and shoes are removed and stored separate from any scrubs used in the facility	0	n/a
Rest Rooms	Toilet facilities directly outside the personnel exit airlock	0	n/a
Storage	Scissor lift or mobile platform to reach high levels for cleaning/	+/(+)	

6 Risk Paths and corresponding Biorisk Control Systems

The loss of control events, namely the exposure of operators with zoonotic biological agents and the release of pathogenic biological agents to the environment are prevented by risk control systems or *layers of protection* that are specific to the identified risk paths for each process. The risk paths fall into 7 categories [26]. The same controls may apply to multiple risk paths within and across categories.

Risk Path Category	Example risk paths	Example Biorisk Control Systems
(I) **liquid**	Facility fabric, drains, no or unvalidated effluent decontamination system, flooding of the facility, sprinkler water release	All potentially infectious liquids, i.e. all liquids from within the biocontainment boundary are considered contaminated and are either autoclaved out or drained in integrity tested pipes to an effluent decontamination system
(II) **air-borne**	Room exhaust air, door ways, leaky facility fabric; drains, soil vent pipes on drains, vents on effluent collection tanks, vacuum line on autoclaves, supply air ducts	Primary containment air tight or with inward directional air-flow (IDA), air change, exhaust air pre-filtration, exhaust air HEPA filtration, effective air change rate, Supply air dampers or HEPA filters, airtight construction of biocontainment barriers and ductwork; ventilation interlocks to prevent over pressurisation; FFP2/3 respirators, PAPRs, Suit-integrated PAPRs
(III) **fomites**	Removal of equipment from containment, contaminated fixtures and fittings in the biocontainment;	Easily cleanable and disinfectant compatible surfaces, seamless construction of bioequipment designed to be cleaned and disinfected, protocols to ensure all materials and equipment being removed from the facility have been steam decontaminated, disinfected with liquid and/or gaseous disinfection process.
(IV) solid waste **(animal carcasses, clinical waste,**	Disposable PPE, animal waste, animal carcasses	saturated steam decontamination autoclave with pre-vacuum pulsing; animal carcasses are difficult to autoclave and should be inactivated in dedicated carcass decontamination system;
(V) animals	Construction permitting	rodent proof barriers; a pest control

	Risk Path Category	Example risk paths	Example Biorisk Control Systems
	(vertebrates and invertebrates that are mechanical or biological vectors)	rodent and arthropod ingress escape.	programme; control of arthropods in barrier facilities with light traps
(VI)	**biological materials**	Inadequate inactivation of samples prior to transfer, transfer of infectious samples to laboratories not equipped or not aware the material is infectious.	Biological materials can only be shipped to biosafety laboratories approved for the biological agents handled in the facility. Material pass through boxes with validated surface decontamination process
(VII)	**people**	Regular users entering/leaving, maintenance people during shut-downs, miscommunication of barrier procedures	Donning, disinfection, doffing discard, change, - water-proof coverall, cut-proof and waterproof aprons/gowns, long sleeved gloves, disposable protective sleeves; positively pressurised sealed suites; powered air purifying respirators (PAPRs);

For each identified risk path one or a combination of several complementing risk controls are applied to prevent the principle loss of control events, exposure of operators and release of biological agents to the environment.

6.1 Guiding Principles for risk control systems

- Contain biological hazards at source as far as reasonably practicable. This reduces the number of different risk paths that have to be controlled: Example 1: confine activity with an aerosolization potential to a biosafety cabinet instead of designating the room as primary containment room for procedures. Example 2: Inactivation of infectious hazards within the facility avoids many additional failure modes and risk paths associated with transportation of infected carcasses and liquids outside the biocontainment facility.

- Hierarchy of controls should be applied to the control strategy, e.g. a practicable engineering control should be given preference over procedural control or PPE [37].
- The ability to decontaminate the facility and all equipment in the facility in a validated and repeatable way without putting the operators at risk.
- Where two risk controls are applied as redundant protection layers for the same critical risk path, they should avoid common failure modes, e.g. if both rely on electrical power at least one should have an uninterrupted power supply. Alternatively, one control is active and one is passive: *Inward directed airflow* is power dependent and airtight construction complement is passive and prevents diffusion if power fails.

6.2 Controls for preventing liquid release

In the general application of the hierarchy of controls, the measures designed into a facility for the safe management of potentially infectious liquids/effluents are an excellent example of engineering controls opposed to “firefighting” with PPE and procedural controls.

6.2.1 Flood prevention and flood protection

Flooding of primary containment spaces can release biological agents to the environment and to uncontained parts of the building. Sources and risk paths need to be considered, e.g. fire sprinklers, utility leaks, blocked drains, taps or hoses left running.

Bunds and ramps to contain floods within the containment area; leak detection in key locations to alarm flood events, “smart” water meter to alarm unusual patterns of water usage, dry 2-stage fire suppression systems, misting systems use a lot less water than regular sprinklers; Water impervious flooring and floor to wall transitions.

6.2.2 Effluent drains

There are many reasons, why effluent drains fail over time, mechanical stress from settling buildings, corrosion by aggressive disinfectants. To be able to demonstrate control it is necessary to demonstrate that drains are not leaking. Effluent drains should not be buried but should be accessible for inspection. If this is not feasible systems to demonstrate drain integrity and alarm drain failures may need to be in place.

Pipe in pipe systems with leak detection systems have become more widely available; they are more expensive, require more maintenance and are much harder to repair than drain runs in an accessible basement.

Drains also have to permit decontamination by steam or by flooding the drains with an effective disinfectant.

6.2.3 Effluent collection system

Effluent collection system should have enough capacity for normal operation and peak usage during campaigns. There should always be enough spare capacity in the collection tanks to keep the facility operational when human error occurs, e.g. a hose is not turned off. The smart water meter and the fill level sensor in the collection tank system should alarm unusual

usage patterns and when the system fills above normal levels before the operation has to be restricted. Effluent collection tanks have to permit validated tank decontamination before the tanks is opened for maintenance. Build up of sludge in the collection tank has to be avoided or cold spots can compromise the tank inactivation. Best practice are cleaning in place systems than permit the tanks to be automatically cleaned at regular intervals.

6.2.4 Modalities for the decontamination of necropsy effluents.

Effluent decontamination systems can use a number of modalities for the inactivation of biological agents. Thermal treatment is the preferred option. Continuous flow systems are more energy efficient and appropriate for larger facilities. However, where effluents contain organic matter like fat, protein and complex carbohydrates build-up of organic material on the internal pipe surfaces can rapidly inhibit thermal transfer and can compromise the hold time at the specified kill temperature due to increased volume flow through pipes of reduced inner diameter. If continuous flow systems are used, they need systems to verify the actual hold time and systems for effective clean-in-place cycles, so that build-up is regularly removed. For smaller systems batch treatment is generally given the preference over continuous flow, but also for high reliability applications batch systems are still preferred.

There is a lot of discussion about the acceptable time/temperature combination. Some argue it must be the same temperature profile as an autoclave, but the directives simply state “validated means”. Most or all of the viruses are inactivated within under 15 minutes at 80°C by more than 6 logs. Even most bacteria are heat inactivated at below 100 °C in under 15 minutes. It is advisable to generate validation data that shows the thermal inactivation in a dose response experiment showing partial kill at shorter times and at lower temperatures. This gives confidence that the treatment is reliable at marginal conditions of system performance.

It is advisable to capture all solids and body fluids at source for disposal as animal waste.

Chemical treatment works well on effluents with no solid content and low bioburden, however, the main concern for chemical plants is the ability of the chemicals to penetrate all particles in the effluent in the treatment time. Chemical treatment can be considered if the active ingredient(s) are permitted under the Biocidal Product Regulations [29] and the chemical inactivation in the largest particles can be guaranteed to be achieved reliably. All particles bigger than 6 mm have to be prevented from entering the drains due to the pre-treatment requirement under the Animal By-Product regulations [28]. Demonstrating the efficacy of chemicals in particulates of different composition is time consuming and newest effluent decontamination systems rely on thermal treatment.

6.2.5 Other regulatory considerations for effluents.

To stay within the effluent discharge consents of municipal sewage treatment works multiple parameters have to be considered. The settleable solid content, the biological oxygen demand (BOD), the chemical oxygen demand (COD), the salt concentrations, and the pH

range are usually regulated with upper and or lower limits. In many cases it is required to install an effluent treatment plant in order to adjust pH or remove solids.

6.2.6 Floor gullies.

Effluent gullies. Trench drains with lightweight covers that are easily cleaned are preferred. The traps should be as deep as necessary to prevent contaminated air flow through the traps, contaminating the necropsy room between campaigns. Bell traps are easy to maintain and do not depend on gaskets to prevent air leakage. Baskets to collect solids >6mm should be sized generously, so they do not have to be emptied multiple times a day. Macerator pumps are not permitted to reduce particles sizes below 6mm [28]. If traps are dependent on gaskets and could dry out, the effluent drains can be maintained at negative pressure to avoid the contamination of secondary containment spaces with contaminated air from the drains. Negative pressure drains can be set up to prove leaktightness of the drains and to alarm if a trap is not sufficiently filled or the gaskets leak and allow air to pass by.

6.2.7 Minimizing the build-up and release of fat

Depending on the nature of the processes a grease trap to EN 1825 shall be considered. They are commonly used in the meat processing industry and in kitchens.

6.3 Controls for Air-borne release and exposure

The controls for airborne release and exposure in the secondary containment spaces are very similar to laboratories and not addressed here in detail. The specific challenges of veterinary post-mortem facilities are the primary containment rooms. The dissection of large carcasses releases droplets and aerosols, the challenge with infectious biological agents will differ widely by the organ, the stage of disease and the techniques of the post-mortem worker.

6.3.1 Engineering Controls for aerosols from large carcasses.

Down draft tables are in used for controlling the exposure to formalin when tissues samples are trimmed for histology. However, downdraft tables generally are not providing any protection when dissecting large animals like cattle, horses, adult pigs or sheep, because the suction is insufficient to capture aerosols and droplets at 30-60 cm above the table surface. With laminar air supply above and suction below, this could be improved, but the large carcass is disturbing the laminar flow and causes turbulences which make it impossible to rely on such a system for protection. The exposure risk and challenge of the PAPRs can be reduced by increasing the air changes in the room and ensuring sweeping air flow from high level supplies to low level returns. In one facility high velocity laminarised air was recirculated back into the room through HEPA filters. This approach which is similar to a grade A air supply in GMP facilities, offers excellent control over air-borne particulates, but requires 300 litres/second of filtered air per m^2. As the reused air does not have to be conditioned it is not as expensive and energy hungry as once through systems, but still a significant investment in fans and HEPA filters. If this feature could just be turned on for the large animal post-mortems, it would augment RPE significantly.

If there is no laminar airflow across the room, but turbulent airflow generated by supply air diffusers there is a potential for infectious aerosols and particulates to deposit at high level and cause exposure during later mobilisation of dust.

Down draft tables and Class I biosafety cabinets may have an application for smaller species, like ferrets.

6.3.2 Containing aerosol generating processes

In all types of bone sawing primary containment is therefore required. Multiple companies offer oscillating saws with vacuum extraction hoods to capture aerosols. Similar devises can also be designed for band saws, but there is no data available as to how much this can reduce the aerosol formation. Pluim [36] recommended the use of a glovebox to capture and effectively extract aerosols at source. Given the aerosol quantities at the most penetrating particle size this appears the best way, which avoids relying on RPE for the protection of operators. At aerosol concentrations that can well exceed the protection factor of PAPR type respiratory protective equipment. One facility had a dedicated downflow booth to protect the operator from the aerosols, but as the airflow was not fully exhausted at low level the down flow booth contaminated the entire area with aerosols negating the benefits of directional airflow at the source. One facility described an exhaust HEPA-filtered booth for the aerosol prone steps and the operator entered the booth in a protective suit with RPE. Selecting the right combination can be challenging as the data to determine the protection factor is not available upfront.

Aerosol control strategy	Advantage	Disadvantage
a) Bone saw with aerosol extraction	Contains aerosol burden at source	Aerosol extraction system requires decontamination process, protection factor not determined
b) Bones saw in a primary containment enclosure with glove ports, e.g. a class I /III hybrid biological safety cabinet	Highest level of operator protection, good for smaller bones and heads	Difficulty to handle the variety of animals in a necropsy facility
c) Booth housing the saw with HEPA filter extraction. Operator enters booth in overpressure suit with RPE	Good protection of the PM room from aerosols.	Operator suit gets heavily soiled. The aerosol concentration may exceed the protection factor of the air filters in the RPE
d) Displacement	Laminar airflow pattern	costly; wide spread

ventilation system in the PM room with high level supply and low level returns.	moves aerosols away from the breathing zone to low level air return terminals.	contamination of the room at low level.

6.3.3 Operator Protection from air-borne exposure

The operators are gowned in personal protective equipment to minimize their surface contamination and aerosol protection appropriate for the task.

Aerosol Control Respiratory Protection systems

Respiratory Protection options have advanced rapidly over the past years. They differ in the protection factor of the air supplies, the ability to chemically disinfect the whole suit and respirator.

System	Approximate Protection factor	
FFP2 respirator mask	20x	CL1-CL2
FFP3 respirator mask	100x	CL2-Cl3
PAPRs with Tyvek suits	1E3-1E4	CL3
Positive pressure suites with a powered HEPA filtered air supply	1E3 to 1E4	CL3
Positive pressure suites with hosed air supply	1E4to 1E6	CL3/4

Only the positive pressure suits with hosed air supply are suitable for entry into highly aerosol charged space.

6.3.4 Aerosol Control Engineering Controls

Barrier	Barrier type	Limitations
Biological Safety Cabinet Class I or II	engineering Control with SOP	
Downdraft Table	engineering Control with SOP	
Effective air change rate in the room	There is no specific minimum air change rate defined. With higher air change rates and a	Proper displacement systems with fan filter ceilings are quite

	displacement ventilation system the operator exposure and the challenge of RPE can be minimised.	expensive and are normally reserved for cleanroom applications.
Displacement ventilation system with low level extraction	RPE active	
Encapsulated positive pressure suits with breathing air supply from hose	RPE	
Encapsulated positive pressure suites self-contained	RPE active	
Powered air purifying Respirator	RPE active	
FFP3 respirator combined with faceshield	RPE passive	
Faceshield	RPE passive	

6.3.5 Air Filters on exhaust or return.

The pathology process uses a lot of water and generates particulates both of which get captured in the room exhaust filters. Conventional HEPA filters lose the microbiological barrier function when they get wet and need to be protected from gross contamination. EPTFE HEPA filters are hydrophobic and thus more forgiving in high humidity applications. G4 and F9 pre-filters are advised at the exhaust air terminals in the primary containment rooms as they cannot be decontaminated in situ in a duct mounted position by gaseous decontamination. They perform >95% of the filtration duty, extend the life of the more expensive HEPA filters and minimise the contamination of ductwork, where the HEPA filters are in a central position. The pre-filters can be changed, when the facility is decommissioned for maintenance, when they are loaded or when a specific hygiene status is required in the facility. For high consequence pathogens that are exotic to the country, generally 2 set of HEPA filters are required in series as a failsafe.

6.3.6 Preventing release via the supply ducts

During normal operation the supply fans are generating airflow and prevent release. Under ventilation failure conditions, room air can be pushed into the supply ducts. For high consequence pathogens The supply ducts can be protected with HEPA filters or spring loaded shut off dampers.

6.3.7 Preventing release via drainage vents, and effluent collection and decontamination systems

Hydrophobic 0.2 µm membrane filters have been established in the pharma industry for decades and have been adopted in these locations, because they are not compromised by moisture and have a better performance than H14 HEPA filters for particles down to 10 nm.

They can easily be decontaminated with steam. They have two caveats: they get blocked by moisture, and they have a significant pressure drop, which may result in drain pressurisation. To avoid condensation on the filters they can be equipped with heating jackets to keep them dry. ePTFE filters could be an alternative where higher airflows are required, but they cannot be steam sterilised.

6.3.8 Preventing release via doorways

The inward directed airflow is provided by a pressure cascade from the clean uncontained areas to the contained and most heavily contaminated rooms. On critical transitions between containment zones airlocks are provided to stabilize the pressure cascade. While more pressure drop results in more airflow across the closed door it is the open door airflow that improves the containment. Door undercuts are a cheap way to provide more directional airflow, but when the ventilation is lost door undercuts also allow free diffusions of dust and contaminants to clean areas. Non-return dampers installed in the door leaf or into the wall above the door only open when a target pressure differential is achieved provide good airflow on open doors and no air reversal when the ventilation fails.

6.3.9 Air change rates.

Laboratories generally have >8 air changes per hour. The higher the clean air delivery rate the more rapid is also the clearance of air-borne infectious particles and aerosols. However, if supply and exhaust terminals are ceiling mounted in a 4-5 m high room, there is a good chance that a high proportion of the fresh air is not reaching the floor or even the breathing space of the operators. Low level air exhaust terminals are providing the best effective air change rate. The necessary air change rate to prevent infection is dependent on source of infectious aerosols, the concentration of infectivity in the aerosols and the infectious dose required.

6.3.10 Laminar air supply.

The best protection is offered by a laminar air supply providing a linear air velocity of 0.3 -0.45 m/s. This is commonly achieved in the microelectronics industry with low resistance fan filter units, but something along this concept has only once implemented in a veterinary post-mortem facility. In rooms with 5 m height this would effectively prevent contaminating the ceiling and the upper parts of the walls, which can only be reached for cleaning with special equipment like a mobile platform or a scissor lift.

6.3.11 UV-C Head space irradiation

Germicidal UV light can also be used to irradiate the head space of the room. This approach is irradiated the air entrained by the supply diffusers in a turbulent mixing system. Regular UV-C must be carefully shielded and managed to avoid operator exposure but recent far UV C (222 nm) technology with germicidal properties and fewer exposure concerns is becoming available.

6.3.12 Air ducts

Decontamination of the facility has to include ductwork and air filters exposed to the room air. If the filters are installed in a terminal position at the room barrier, the decontamination of the ductwork is not critical, but it may still be necessary to circulate the gaseous decontaminant through the ductwork to inactivate the filters or distribute it in the room. Galvanised ductwork is most commonly used, and can withstand corrosion by H_2O_2 vapours. However, it also acts as a catalyst and degrades VHP rapidly [38]. Ductwork intended to be decontaminated with VHP or to distribute/recirculate VHP should be made of welded stainless steel.

6.4 Controlling release and exposure by fomites

Inanimate objects that can mechanically transfer infectious agents are commonly referred to as fomites. In the post-mortem facility fomites are the tools, and the equipment, but could also be the facility fixtures and fittings. To prevent fomite mediated risk paths, equipment used in the facility has to be designed so it can be decontaminated effectively for the infectious challenge it may encounter. The same is true for all surfaces, fixtures and fittings in the facility, all have to permit effective decontamination.

6.4.1 Decontamination autoclave

The Preferred decontamination is steam autoclaving, because it is very reliable and widely accepted. To be effective it is a combination of heat and the chemical reaction of collapsing steam with organic material. Decontamination autoclaves should have a prevacuum to remove all pockets of air with steam.

6.4.2 Low temperature steam (formaldehyde) sterilisation

Many materials and even electronics are heat sensitive and do not tolerate high temperatures of steam autoclaves. Special autoclave cycles, which require custom designed autoclaves treat sensitive equipment with low temperature steam in a deep vacuum at 50-70 °C [39]. This can be combined with formaldehyde or hydrogen peroxide [40]to achieve sterilisation. This process permits the reliable inactivation in equipment cavities that are not hermetically sealed.

6.4.3 Material Airlock

Material airlocks are used for the decontamination of heat sensitive equipment. The equipment is manually cleaned with detergent or a detergent containing disinfectant to remove organic contamination. Once the equipment is cleaned, it is wipe disinfected. Gaseous decontamination is a final step to reach into crevices ensure 100% of the surfaces have been reached without soaking the items in disinfectants.

6.4.1 Tools and disposable items

Tools used during the necropsy have to be cleaned and decontaminated before they can be brought back to the clean supply room. As blood and proteins bake on during autoclaving, a chemical decontamination/cleaning may be advantageous before autoclaving but manual

cleaning of sharps should be strictly avoided. Partswashers can achieve decontamination and cleaning in one step.

6.4.1 Laundry

Contaminated laundry should not leave the containment area untreated. be autoclaved, but used not visibly stained laundry can be washed in the facility at high temperature. >70° C for >30 minutes. If it is laundered off-site it should be autoclaved prior to shipping. The location of the laundry machine needs to be considered from multiple angles. (1) Loading contaminated laundry, (2) unloading clean laundry without recontaminating it, (3) potential breakdowns of the laundry machine, contaminating the room. To clean soiled PPE and extend its life, the fabric is directly laundered without prior autoclaving. This is acceptable for most viral and bacterial agents apart from highly heat resistant bacterial spores. To prevent the contamination of the laundry room during loading the soiled PPE packaged in water soluble bags made of polyvinyl alcohol material, which dissolves above 60 °C but provide an adequate biological barrier at room temperature, even when they get wet. The bags are collected, surface disinfected before transport to the laundry room. Laundry drainage must be directed to the Effluent decontamination system. Industrial laundry machines have better temperature control, some laundry machines for domestic use are not able to reach the chosen temperature across the load. Regular temperature verification with temperature loggers can help to (re)validate the washing temperature. Ozone generators can also enhance the decontamination process at lower temperatures and are routinely used in hospital laundries for infection control.

6.4.2 Facility decontamination and decommissioning

Designing cleanability into the surface finishes must be coordinated with accessibility, furniture and fittings that enable cleaning and disinfection. To reach the ceilings in a 5m high PM room requires mobile platforms. Where are these stored? One strategy is to minimize the contamination of the head space by ceiling air supplies and low level extraction. Minimizing aerosols in the room procedure and engineering controls that capture aerosols at source. The head space of the room is is kept clear and can be treated with gaseous decontamination or design permitting with UV-C irradiation.

If gaseous decontamination is planned to reach inaccessible surfaces the same principles apply as in other primary containment spaces. Gaseous decontamination can only inactivate traces deposited by fine aerosols, it is in principle not suited to decontaminate any gross contamination, it requires room seal ability, air circulation to reach all surfaces. Diffusion into hollow objects, pipes and ducts does not suffice to achieve decontamination. So all hollow spaces need to be sealed in such a way that they do not get exposed or need to be included in the cleaning disinfection regime, and the validated gaseous decontamination process

6.5 Other potentially infectious waste

6.5.1 Clinical waste

Disposable PPE, e.g. Gloves packaging etc. can be treated by decontamination autoclave. Standardised package sizes help to work within the validation parameters used for the autoclave. Important is that decontamination autoclave design and validation is applied.

6.5.2 Chemicals

Chemicals that cannot be autoclaved for disposal, may need to be inactivated by another treatment before being shipped to a hazardous chemical waste service.

6.6 Disposal of carcasses and animal tissues including blood

Under the controls of the animal by-product regulations carcasses and animal tissues need to be treated and disposed by an approved method [27,28]. Where hazardous biological agents are intentionally handled, e.g. in infectious disease studies, the biological agents directive[14], the GM Directive [15] and relevant controls for hazardous animal pathogens apply and require inactivation of the biological agents of concern prior to shipping the carcass material to an animal by-product processing or incineration facility. Carcasses from infectious experiments are by definition category 1 animal by-products. Incineration is the main terminal disposal route, but several methods are approved under 2011/142 for the prior treatment of animal by-products, which can reduce the containment requirements on remains from the treatment shipped to an incineration or co-incineration plant.

It is recommended that animal carcasses and other animal by-products are inactivated prior to leaving the biological containment and prior to shipping the safe residues to a licenced carcass disposal facility. The inactivation of carcass material is best achieved by heat. Steam autoclave cycles take long and often fail to reach temperature in the carcass core.

Methodology	Pros	Cons
Incineration	Final disposal method per EC 142/2011. Can also handle bedding from animal rooms	Difficult to integrate into biocontainment barriers, due to dynamic airflows. Strict emission controls for clinical waste incineration [41] are difficult to achieve with small mixed loads Highest Cost of operation (unless used for energy generation)
Rendering	Recognised Treatment option under EC 142/2011 Well established in many	Most systems are for larger volumes and may not operate with small loads.

		high containment facilities. Safe inactivation of all biological agents other than prions. Some can also be used for bedding. Wet of dry end product	
Alkaline Digesters	**Hydrolysis**	A recognised method for carcass inactivation under EC 142/2011. Wet or dry end product	The residue is very high in salt and may require special precautions for incineration. Bedding is generally not digested and can only be processed by some units
Autoclaves – as a back up		Autoclaves are standard equipment and are a requirement for containment facilities Acceptable for inactivation of laboratory rodents	Not a reliable method for livestock carcasses. Validation is the most time consuming Requires dismembering carcasses as radiant heat penetration of carcasses is not effective. Not an approved treatment under EC 142/2011. Carcass material has to be shipped as category 1 animal by-product for incineration

6.6.1 Off-site transportation

Once inactivated the carcass residues can be shipped as animal by-product to a licensed facility. At this point the material is no longer infectious and thereby not subject to the ADR [30]. This has a significant impact on the cost of transportation. The ADR regulations permit the transport of animals infected with epizootic disease agents (UN2900, UN2814, UN3373) in leakproof bulk containers, but this is understood as a concession to enable transport during epizootics rather than approving the transport of animals from infectious disease research.

6.7 Animal Risk Path Controls

Inadvertent ingress of vertebrates and invertebrates can result in infection or mechanical contamination of these animals. All reasonable measures have to be taken to control rodents and other vertebrates by ensuring building is rodent proof. This can be achieved with a tight

building construction and tight fitting doors that have no undercuts. To control invertebrates is more difficult and will require a combination or measures, but will require an active pest control programme.

6.8 Biological Materials

Biological materials have to be submitted to other diagnostic laboratories and collaborators all the time. It is important that the recipient understand the range of biological agents potentially handled in the facility and have corresponding controls and authorisation to handle materials received. In many cases materials can be rendered inactive for genetic analysis or biochemical tests, Protocols that preserve the biological properties relevant for analysis but reduce or eliminate other biological hazards are a foundation of successful exchange of materials. The material transfer agreements should ensure the sender and the recipient are fully informed of the potential biological hazards and are authorized to accept these.

6.9 People

The main challenges managing people related release and exposure is down to human factors. How to align the procedures steps between operators so contamination does not spread by inconsistent procedures working in primary containment, working in biological safety cabinets and decontaminating/degowning in barrier facilities. Facility lay out can support users to sequentially carry out procedural steps that have a necessary sequence, and using standardised steps defined and trained by SOP. There is no “one fits all rule”, but a thorough task analysis considering the least motivated and trained operatives will help to shape the right layout and avoid that procedural steps are skipped simply by error.

6.9.1 Hand wash stations and hand sanitizers

There is a great deal of misunderstanding between users and designer/ architects as to what constitutes a fit for purpose handwash station. The hand wash process should be contact free (faucet, soap dispenser) under running water (no plug). Sink and faucet are designed so the hands, wrists and lower arms can be lathered with soap and rinsed under the faucet without touching the faucet of sink and without splashing out of the sink; this is achieved by faucet design, sink size and sink design. Best in class are surgical scrub sinks, but clinical hand wash basins can also be used. Ideally, the handwashing results in zero splashes on the floor. Hand sanitizers can complement Handwashing but are not a substitute. The following table lists user requirements for hand wash stations. In critical locations paper towels are the preferred mode for hand drying, they reduce the residual bioburden on the hands better than electrical hand dryers. In less critical applications hand dryers may be used as they are more sustainable.

	Safety Attribute

UR01	**Basins** should have a smooth form and easily cleaned surfaces, above and below. Ideal materials are high quality ceramics with lotus effect (e.g. CeramicPlus® or Keratect® and stainless-steel fixtures.
UR02	Minimum **basin dimensions** 350 mm front to back × 500 mm wide. Sink rim should be 900 to 950 mm above the floor
UR03	**Basin overflows** is not provided for infection control reasons.
UR04	Medium or large **integral back-outlet** basin with no plug is recommended. (back outlet show less splashing than regular outlets) If necessary a standpipe shall be provided to fill sinks.
UR05	Taps are required to be operated **handsfree**. Sensor-controlled taps are ideally suited to control the flow of water and can offer the additional benefits of controlled run times and automatic purging / hot water pasteurization to mitigate stagnation and biofilms. Lever-action elbow taps can be accepted where technically or operationally justified.
UR06	Wall mounted faucet minimum 260 mm distance from basin floor
UR07	single **self-draining** faucet avoids stagnant water in the faucet
UR08	Flow regulators may be required to soften, shape and direct the water jet from the faucet. Laminar flow regulators are preferred over aerating ones as they generate fewer aerosols.
UR09	The water jet from the tap should not be directed into the sink outlet to avoid contaminated splash back.
UR10	Thermostatic mixer tap approved for hospital use
UR11	Wall mounted handsfree soap dispenser and paper towel dispenser
UR12	Wall mounted hand sanitizer

6.9.2 Boot decontamination

Boots should be disinfected as frequently as necessary to avoid spreading contamination within the facility. Basins with disinfectants can be useful visual clue, but should not become a trip hazard.

6.9.3 Step over benches

Step over benches or other clear intuitive demarcations separating clean from potentially contaminated floor zones in the same room can support users, but still have to enable emergency egress from the area.

7 Definitions

7.1 Biological Agents [42]

Any microbiological entity, cellular or non-cellular, naturally occurring or engineered, capable of replication or of transferring genetic material that may be able to provoke infection, allergy, toxicity, or other adverse effects in humans, animals, or plants. For the purpose of this document, prions are regarded as biological agents.

7.2 Adventitious biological agents

Biological agents that are present in the animals studied, but not intentionally handled. They are a concern in so much as they pose a hazard to the workers, the environment, or adversely affect the outcome of the research studies. SPF animals will be selected to avoid such biological agents in the source population. The adverse effects may also be controlled by vaccinating animals early so their immunity will have eliminated the adventitious agents of concern. Adventitious agents have to be considered in the facility risk assessment as these biological agents encountered unintentionally may pose a higher risk than the biological agents under study. Based on the regional prevalence of significant zoonotic biological agents study animals should be sourced from suppliers with known health status. If this is not feasible, vaccination should be considered for animals and operators to reduce the likelihood of work related infections with zoonotic agents.

7.3 Primary Containment

Wherever technically feasible the biological agents are contained at source in primary containment. This may consist of a biosafety cabinet with appropriate procedures at the open front, a downdraft table, the pressure vessel used for carcass rendering, or the kill tank system used for effluent decontamination. In many cases, with livestock species, the necropsy in primary containment devices is not feasible and the necropsy room forms the primary containment. If activities can be consistently carried out in primary containment the room serves only as secondary containment.

7.4 Protection layer

Any biological risk control system that prevents the loss of control or the escalation following a loss of control is referred to as a protection layer. Protection layers are characterised by their protection factor, reliability, availability to name some key criteria.

7.5 Decontamination

Decontamination refers to a process that renders the biological agents of concern non-infectious. It differs from sterilisation that it is focussed on inactivating the highest

concentration of a target organism or set of target organisms. In many cases it may require more than a 6 log reduction, but in many cases it can be achieved at temperatures well below the 121 °C standard sterilisation conditions. In most cases sterilisation conditions are used because there are well standardised validation protocols available. The vast majority of biological agents are already most heat sensitive than *Geobacillus stearothermophilus.*

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Annex 1 Process Map for a biocontainment animal facility working with zoonotic and exotic biological agents in livestock species.