

Regulatory Compliance of a BSL-3 Laboratory Unit in a Drug Discovery Environment

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Abstract

This publication describes the implementation and integration of a biosafety level 3 laboratory (BSL-3) unit within a drug discovery environment into a public multi-tenant and multi-user complex of buildings. The manuscript is intended to be used as guidance for any owners/users willing to build a laboratory unit of this type, including all steps from design to registration and certification.

The goal of integrating a BSL-3 laboratory, according to international standards, with a drug development unit and an animal facility for infection studies, in a mixed multi-user and multi-tenant building, under circumstances where biosafety legislation is still under development, can lead to a complex situation with conflicting demands and expectations of the involved parties and the public. To solve the potential conflicts, an innovative approach was chosen to optimize the design of the laboratory (basing the safety concept on user requirements and qualifications criteria, and developing technical solutions in compliance with a consensus of the most relevant international biosafety regulations).

We describe here the BSL-3 unit set-up, built for the Novartis Institute for Tropical Diseases (NITD), which moved in April 2004 to Biopolis in Singapore, where new laboratories compliant with international biosafety standards had to be set up. The project ran in six phases; after 14 months of construction and build-up, installation approval, commissioning and certification, the laboratory could be made available to users. After evaluation and approval of all Standard Operating Procedures (SOPs) with non-infectious agents, the laboratory could be used as a so-called "hot operation" with infectious agents.

Introduction

The intention of this publication is to provide guidance to future users intending to set up BSL-3 laboratories in a drug discovery environment and to assist them during the procedures of construction, commissioning

and certification. The construction of the NITD BSL-3 unit is provided here as an example of how to translate the recommendations into reality. In April 2004, the Novartis Institute for Tropical Diseases (NITD) moved to Biopolis, an integral part of the life sciences cluster in the "one-north zone" (www.one-north.com) in central Singapore. This area encompasses approximately 500 acres that will be turned into a base for research and development, commercial, residential and recreational activities over the next 20 years. The new research laboratories of NITD, including a facility for small animal experiments, are located on the fifth through eighth floor of the Chromos building at Biopolis. For the realisation phase, a supervision team (namely the authors as a core group) with representatives of the owner, users, and consultants was created. The supervision team subdivided the project into six different phases. The first part dealt with a comparative overview of biosafety related legislation in the U.S., Canada, Europe, Switzerland, and Singapore (draft) as well as the World Health Organisation (WHO) standards.

In the second and third phase, based on this overview, we gave recommendations for the planning, design and realisation of the BSL-3 laboratory unit, including the development of user specifications based on user requirements. In the fourth and fifth phase, user specifications were transformed into individual technical solutions with corresponding commissioning activities. The last phase was completed with the process of certification. In Singapore, the relevant legislation was still in development. For this reason, the establishment of laboratories of higher security and safety levels in Biopolis can be considered "a model project" that may serve as a reference for the development of Singapore's biosafety and biotechnological research community.

Objectives

The first objective was to develop a biosafety concept on a technical and organisational level. Then, our goal was to collect and compile directives and operating regulations for the safe operation of the BSL-3 laboratory. We based these rules on a comparison of current interna-

tional biosafety legislations. The subsequent objectives were to draw the line from the recommendations to the realisation of the facility. We then evaluated the implementation in comparison with the specifications during the installation approval. The system was qualified during the functional approval and validated during the operational approval. Finally, we needed to constitute the conditions for the certification of the laboratory before switching to the so called “hot operation” mode.

To achieve these goals, biosafety standards and working practices had to be defined that complied with a consensus of local, country-specific and international and local regulations and guidelines. The follow-up objective was to provide information regarding the specific user requirements for:

- Design and construction of the laboratory
- Technical equipment
- Organisational measures including safety and security guidelines

This integrated approach is not only a collection of international ordinances, but also the basis for creating a state-of-the-art effective research environment providing a high safety level.

Biosafety

General Requirements and Considerations

Modern biosafety legislation is directed at the protection of workers as well as protection of the public and the environment. The fundamental assumption is that the unintended release of pathogenic or genetically modified organisms might exert major harm or, at the very least, pose non-quantifiable risks to human beings and the environment.

Consequently, the central principle of biosafety is the containment of microorganisms to prevent their unintended release. The current understanding of modern biosafety legislation is that all pathogenic, or genetically modified organisms have to be effectively blocked from release from the laboratory or facility during handling. This is a completely different view as compared to the classic hygiene related fields such as infectious diseases. In the latter, minimization of release, or inactivation below a critical infection dose is the most relevant requirement. In contrast to this—as a hypothesis—modern biosafety legislation, as it is understood by many biosafety experts in Europe, bases its approach on the assumption that organisms with new properties may wreak havoc on humans and the environment. This holds true only as long as very little is known of biological safety research and no better data exists on the real risks of the release of pathogenic or genetically modified organisms.

Containment Principles

“Shell-in-a-Shell” Principle—Measures of containment have to restrict the spreading of microorganisms to

a well defined volume. This could be a tight flask, tube or container for handling the organisms. The wall of the flask is not only a liquid enclosure, but also the first line of defence. In addition, if this first line of defence is removed by breaking the flask, there must be another containment volume encompassing the inner one to contain the microorganisms from unintended release. This is called the “shell-in-a-shell” principle.

Each shell has a certain probability of failure. The probability of an installation, or available detection method for failing, will determine the degree and number of safety measures to be applied to rule out any release of organisms into the environment. In this view, one could draw the false conclusion that the risk of unintended release of organisms could be significantly reduced by using additional containment shells.

Therefore, the question is how many containment shells are technically needed to provide safety? Obviously, the outer shell will have a greater volume than the inner one, leading to a certain rate of distribution and dilution (volume expansion) of organisms in case the inner containment volume fails. In the case of one individual shell failing, one should be able to prevent a release of organisms with the help of the negative pressure cascade. If the pressure cascade also fails, a retrograde airflow into the inner shell will prevent any release until pressure equilibrium is reached. In the state of equilibrium, no exchange of air will happen. Minor aerosolic contaminations can be minimized and removed by air exchange. To protect workers from contamination, it may be—as another hypothesis—sufficient to lower the contamination level by dilution, or inactivation until a concentration of the pathogen well below the critical infectious dose is achieved. In an analogous manner, numerous agents for disinfection do not achieve sterile conditions, but reduce the level of viable infectious organisms, which in effect will be below the critical infectious dose.

From the viewpoint of the legal “zero release” requirements, such as Genetically Modified Organisms (GMO) things may look different. Theoretically, a “zero release requirement” calls for an unlimited number of shells. Such a demand cannot be met under realistic circumstances.

The minimum detection level (by modern detection methods like MALDI-TOF mass spectroscopy) for organisms limits the number of shells required to maximise the containment effect. Generally, a statistically low failure probability for a single containment shell also rules out the necessity for a higher number of shells. In practice, three elements of containment can be found in a standard level-3 laboratory: the container for the organisms, the biosafety cabinet (BSC), and the laboratory suite itself.

Containment shells are sensitive to damage at the interface to the outer environment. The integrity of following systems might be affected:

- Construction elements of the shell

- Walls and their coatings
- Ceiling space
- Airlock
- Windows
- Material pathways
- Utility penetration points (Figure 2)

In the example presented in this publication, the highest attention must be paid to the ceiling space. The ceiling space is designed as a service area that contains a high number of shell penetrations and installations.

Incident Probability and Redundancy in Hazard Prevention—Technically speaking, the perception of risk of a possible hazard is defined as the product of the probability of an incident and the impact of its consequences. This definition differs from the risk perception by the public, which can be significantly determined by emotional factors. Technical safety measures are subject to

failure in a wide range of probabilities.

Technical failures can be compensated for with redundancy in implementation and the appropriate design of control measures, which also might be redundant in itself. Compared to human errors and failure rates, technical failures can be considered to be at the low end of the incident probability scale. In this view, human errors have considerable weight in the estimation of hazard incident probability. For this reason, intelligent safety design also tries to compensate for human errors on the entire operational safety of a system.

Technical Measures versus Organizational Measures in a Safety Concept—Generally a safety concept is understood as the theoretical description of all measures that are operating to establish the containment. As a rule, technical and organisational measures have to complement each other. Both aim to achieve high operational

Figure 1

Dengue fever lab—individual ventilated cages (IVC) and biosafety cabinet.



Figure 2

a) Cable penetration with multiple cable transfer unit (MCT); b) Cable penetration with gland.



safety and reliability.

Whether the balance between these two influences tends to give weight to technical measures, or to organisational measures, depends on many independent factors such as budget, level of assessed risks, amount of enclosed material, required effectiveness in material handling and many others.

Release Paths—Microorganisms can find their way out of their containment using different paths, which are:

- By air, as an aerosol,
- By media (waste water, as a contaminant)
- By vector as a contaminant (human beings, animals, material transport, operational failures), and
- By intended transport of material with potentially damaged containers.

Worker's Health Protection—Worker's health protection is a main topic of biosafety for two reasons. First, workers have to be protected from any risk posed by the organisms they work with. Second, the worker himself may play an important role as a vector. The rules for workers protection are an integral component of the biological safety concept and complement the rules of good microbiological practices. They also have to comply with the local biological protection laws.

Animal (Product) Protection—The protection of test animals is a third important aspect of biosafety besides the requirements of workers and environmental protection. The necessity of maintaining Specifically Pathogen Free (SPF) conditions in livestock breeding demands a high attention to contamination control.

To investigate the mechanisms of infection for pathogenic organisms, infection experiments must be tailored to be monocausal in as many aspects as possible. For this reason, animal pathogen research relies primarily on the use of SPF-test animals or, at least, animals maintained under Optimized Breeding Conditions (OBC). The latter—with a sort of standardized biodiversity in their intestines—are kept under conditions designed to prevent intrusion and spreading of any pathogens within the facility. Although SPF and OBC breeding facilities have completely different protection goals as compared to infection laboratories, the technical preconditions are often very similar in their underlying principles. Despite the potential conflicts, both types of installations often have to be integrated in close neighbourhood for economic reasons. Therefore, high attention to the issues of cross-contamination prevention is required.

Cross Contamination Prevention—In our approach, cross-contamination prevention is indirectly linked to biosafety concepts. Unfortunately, biosafety legislation does not address this topic. However, all measures that are suited to control, or prevent the unintended release, or spreading of organisms ultimately benefit the scientists' work and favour the concepts of production safety.

Environmental Protection—In modern biosafety legislation, environmental protection is meant to work

towards an absolute impenetrable barrier to any genetically modified or pathogenic organism. Regardless of whether this view can be supported by any risk assessment or not, the public's expectations on safety require that containment measures are one hundred percent effective.

Biological Monitoring—When handling pathogenic Genetically Modified Organisms (GMOs), the risks posed by these organisms are defined individually by the properties of each specific pathogenic strain. In the understanding of the authors, a significant change in the properties of an organism like the pathogenicity conforms to the generation of a new substrain. Therefore, recognizing individual strains by their genetic properties is a prerequisite for safe handling. Consequently, tools for the identification of each individual organism on the strain level must be at-hand. Available detection methods, such as the latest developments in MALDI-TOF mass spectroscopy, or immunochemistry, together with standard routine test procedures, which are defined in the biosafety concept, are the basic tools to be used in control of all potential release paths and provide the means for the identification of the organisms.

Quality Assurance—In the opinion of the authors, quality control and quality assurance for biosafety must encompass all means and measures required to safely handle and contain the organisms of interest. Quality assurance in biosafety also has to ensure that all handled organisms are known as individual strains. Furthermore, quality assurance does not stop at the interface of the containment to the outer world, but also includes transportation and academic exchange of organisms. As a consequence, all organisms underlying intended use have to be accompanied by documents containing all of the necessary information for their safe handling and individual identification.

Documentation—Documentation must encompass all relevant information necessary to allow for the safe operation of the facility. Technical equipment, as well as all organisational means and measures, must be documented. In general, written records of the following topics must exist:

- Work procedures and Standard Operating Procedures (SOPs)
- Maintenance guides and replacement schemes for relevant installations that affect safety
- Safety concept (e.g., the [seamless] interaction of all technical and organisational safety measures as well as a management commitment to safety)
- Lists of organisms in use and storage with information on their individual properties;
- Hierarchy scheme of responsibilities, competences and authorizations
- Safety training and respective education documents
- Workers health protection documents, or at least reference links to them

- Action plans in case of an emergency, release or spillage
- Major accident and fire prevention schemes
- Major accident coverage plans
- Alarm schemes of different levels in the organization
- Security regulations
- List of registered personal and permissions

Security and Biosafety—Security is an integral part of a biosafety concept. Security measures shall provide the means for secure and uninterrupted operation of the containment and ensure that only authorized and competent staff has access to dangerous material.

Badge Access Control and Interlocking Doors Keypad—Two security barriers control access to the containment area: One system consists of authorization of access rights assigned by responsible and trained staff for the BSL-3 laboratory and site security. The second is an interlocking system provided with a keypad through which authorized people can access the facility with a digital access code that is personalised and changed on a quarterly basis.

Holding Area (Vestibule and Dressing/Undressing)—Staff members change their lab coats with one-way overalls (bunny suits) in a holding area before entering the experiment area. It is also equipped with a shower room that can be used when leaving the laboratory. The shower room can also be used to treat chemical spillage in case of an accident. From this area onwards, the whole laboratory is under negative air pressure.

Recommendations and User Requirements

We base our recommendations on the comparative overview of national and international legislation and guidelines (Table 1). A close cooperation between scientists, management and suppliers is essential to achieve a well-elaborated design proposal that is in compliance with user requirements. For the determination of the final design, it is important to integrate strategic management decisions with future action plans and research activities. Some key decisions such as final lab layout and future research plans, are needed to determine the advancement of the project development. Not addressing these topics at an early project stage could lead to abortive design, lost time and could further jeopardise a successful project, its financial plan and timely construction completion.

Design and Concept of the Laboratory

The following collection of requirements, rules, and guidelines reflect the requirements of international biosafety legislation. On the other hand, they are, in their individual compilation, an adaptation to circumstances in a multi-user environment. In the design of the laboratory, a major emphasis was placed on a moderately high degree of redundancy in critical installations that affect safety in

order to provide a continuous (24/7) safe operation. We have set up this design in order to allow for continuous operation even under circumstances when parts of the installation have to be set out of order for maintenance purposes. This way, down times can be effectively limited to rare cases of emergency.

Separate Work Area—A separate work area, as it is required by Swiss legislation, has to be understood as a work space with limited access dedicated to level 3 activities. This is one of the most basic requirements in international biosafety legislation. It is a precondition for effective control of personal access and material transfer and allows for an efficient application of the rules for safe microbiological laboratory practice while minimizing the probabilities of cross contamination and unintended release. Therefore, this feature is implemented in the basic design of the laboratories at the outset.

Airlock—Access to the containment area must occur via an airlock with interlocking doors. This is also a basic requirement, which, in combination with a badge access control, will guarantee the integrity of the containment by preventing the unintended release of potentially contaminated aerosols through the access area. Additionally, it provides the means for access control as well.

Rodent Barriers—Rodent barriers and pest control measures must be present in the laboratory as part of the hygiene concept. They must prevent the escape of infected animals from, and the intrusion of wild animals into the restricted area.

Observation of Work Area—An observation gallery alongside the laboratory for visitors as well as for security checks and glass windows between the laboratory rooms are recommended to be installed so that about 75% of the facility can be visually monitored from outside the BSL-3 zone (Figure 3). An additional CCTV system completes the security measures.

Resistant Surfaces—Most modern standard laboratory benches and furniture are sufficient. They must have non-porous and non-absorbent surfaces, such as glass, ceramics, and synthetic material with appropriate acid and alkali resistance. Cavities and hidden surfaces that are not easily accessible for cleaning have to be avoided.

Cleaning and Maintenance—Floor corners have to be “smooth” in order for easy cleaning and sanitation. Smooth and slip-preventing floor material also facilitates daily cleaning.

Liquid Waste and Waste-Water Treatment—With a so called “semi-dry” sanitation concept, no floor drains need to exist in the laboratory area. All effluents, including collected spills, leaving the containment laboratory area have to undergo waste water treatment. Since waste water from cleaning and sinks is a potential source of contamination, it must be retained in the containment area until inactivation. Floors have to be designed accordingly with 10cm-retaining-water barriers at door openings and a removable water barrier in the airlock (Figure 6).

Table 1

Overview on international ordinances and guidelines (as per July 2003).

Country	Building/Purpose	Protection at Work	Transport	Import/Export
USA	CDC/Office of Health and Safety (OHS) Biosafety in Microbiological and Biomedical Laboratories set guidelines for laboratory biosafety.		CDC/Office of Health and Safety (OHS) Biosafety in Microbiological and Biomedical Laboratories set guidelines for laboratory biosafety. Biosafety and transport is regulated by the CDC/OHS Interstate Shipment of Etiologic Agents Regulations and the Department of Transport Hazardous Materials Regulations.	The Export Administration Act (1979 amended 2001) prevents U.S. companies and individuals from exporting any goods or technologies to certain countries that would directly and substantially assist a government or group in developing or delivering biological weapons. Code of Federal Regulations (CFR) 71.71443 requires that a permit be obtained from the CDC for importing etiologic agents of human disease.
Canada	Health Canada also publishes Laboratory Biosafety guidelines.		The Transport of Dangerous Goods act (1992) requires that all dangerous goods be properly contained and marked. The Transport of Dangerous Goods act does not require registration for transit of dangerous goods.	
EU (with Germany as an example)	Law order on the regulation of gene technology (GenTG, 1993, BGB1. 1S. 2066) and Gene technology safety ordinance (GenTSV, 1995, BGB1. 1S. 297*	Parliament Directive 2000/54/EC and Council Directive 90/679/ECC (1990, amended 1993, 1995, 1997) -> protection of workers from exposure to biological agents.	Council Directives 93/75/EC (1993 amended 1997, 1998), 94/55/EC (1994, amended 1996), 96/49/EC (1996) regulate and harmonize the laws on transport of dangerous goods by boat, road, and rail respectively.	Council Regulation (EC) 1334/2000 (2000, amended 2001) -> regime for dual use items and tech. The Regulation requires each member state to develop legislation licensing or prohibiting export of certain dual use items.
Singapore	"OSH Guidelines for Laboratories and Production Facilities in the Biomedical Sciences Industry"; Ministry of Manpower; draft, downloaded June 23, 2003		Guidelines on the import, transport, handling, and disposal of human pathogens for diagnosis, scientific research, and industrial uses in Singapore, Quarantine & Epidemiology Department, National Environmental Agency, April 2003.	Singapore
WHO	WHO World Health Organization, Laboratory biosafety manual. - 3rd ed. 2004 (WHO/CDS/CSR/LYO/2004.11)		International regulations for the transport of infectious materials are based on the biennial recommendations of the United Nations Committee of Experts on the Transport of Dangerous Goods. WHO World Health Organization, Guidance on regulations for the Transport of Infectious Substances, September 2005 (WHO/CDS/CSR/LYO/2005.22)	

Figure 3

a) Observation gallery from inside the BSL-3 laboratory; b) Glass windows between the laboratories inside the BSL-3 zone.



Figure 4

Liquid nitrogen supply from outside the BSL-3 laboratory.



This way, any spills or water ejected by sprinklers during an alarm situation can be retained for chemical or thermal inactivation treatment. Alternatively, sinks with a built-in thermal “inactivation flow through device” may be applied, but in the present case, a decision was made for a centralized inactivation installation for economic reasons. Generally, any waste water and liquid waste must be inactivated chemically or thermally.

Media Supply—Regarding media supply the following aspects have to be considered:

- Media ducts must be sealed by using compressed rubber sealants.
- All media and power lines that supply the BSL-3 perimeter have to be disengageable from outside the laboratory (Figure 4, the liquid nitrogen supply).
- All liquid media must be equipped with one-way valves to prevent back flow.

- The design must be leak proof.
- There must be a leakage monitoring system (electronic liquid detectors) in all critical areas, (under water-cooled devices such as centrifuges, freezers, and biosafety cabinets).
- All circulated media, such as cooling devices, must be designed as closed systems, if necessary, with a heat exchanger.
- Sealable doors with two panels (1.4 m [w] x 2.10 m [h]) for roll-in and roll-out of larger lab equipment) are highly advisable.
- Showers are optional and can be available as a safety shower in the dressing air lock. If showers are present, waste-water must be collected and inactivated appropriately.

Air Handling and Pressure Cascading—Negative air pressure has to be maintained permanently in an uninter-

Figure 5

Pass-through autoclaves of a BSL-3 laboratory.



rupted 24/7 operation mode. To assess the risk of a technical failure, pressure has been considered in decreasing levels from areas of moderate risk (corridor, i.e., -25 Pa) to areas of higher risk (animal rooms, i.e., -65 Pa) to force directed airflow. For the worker's comfort, air velocity should not exceed 20cm/s. Back flow, oscillations and noisy air movements are prevented by careful balancing of supply and exhaust air.

The system must offer the option to isolate any specific compartments as well as to control the individual volume flows and pressure levels at any time.

High Efficiency Particulate Absorption (HEPA) Filtration—The exhaust air shall not be recirculated to any other building area and has to be discharged to the outside via HEPA filters. If chemical fume hoods are needed within the BSL-3 lab, exhaust air has to be HEPA-filtered, too. This can be achieved with the installation of a biosafety cabinet (class II B2, which is hard-ducted), often called a “workstation” in the European market. Such “workstations” are offered by various suppliers.

Air Conditioning—The air-conditioning units in air handling supply lines lead low turbulence air into the laboratory, maintaining stable conditions with respect to temperature and humidity. In practice, significant amounts of waste heat can be removed from the laboratories without forming condensates on cooling surfaces. In

this manner, working and living conditions (for animals) are kept favourable, and electronic equipment is protected from detrimental moisture. We recommend increasing the air exchange rate in the front of, and behind, autoclaves in order to minimise humidity and to remove unpleasant odours. An increased air exchange rate is also advisable in the air lock as a safety measure, because contaminated aerosols could accidentally be generated inside, or near the air lock. During the air exchange process, the doors are interlocked. In case of an emergency, the interlocking door principle will be overrun automatically to allow for unhindered exit of the laboratory. This procedure is required by the owner based on a current interpretation of the Swiss biosafety ordinances and is accepted by the Singaporean legal authorities.

Technical Equipment

Solid Waste Treatment—According to the WHO, autoclaves or incineration systems must be available in the laboratories. In particular cases, chemical treatment may serve as an alternative. If so, an individual SOP must be integrated into the safety concept. Nevertheless, there must be room for temporary storage of waste within the facility and without constraint for internal traffic, or free access to emergency escape routes.

Prevention of Aerosol Formation—To avoid aerosol

formation, the following conditions and devices should be identified and installed:

- Centrifuges with aerosol-tight rotors
- Homogenizers designed as closed systems (using mini devices with a lid)
- Fluorescence activated cell sorting devices (FACS) will make individually designed measures for the removal of aerosols necessary
- Microscopy of living higher-risk-level organisms also demand individually designed measures
- Double containment of high-risk microorganisms at all times outside the biosafety cabinets (BSC)

Instruments and Equipment—The entire BSL-3 laboratory area has to be equipped with materials and instruments dedicated only for its rooms and specific purposes.

Refrigerated Storage—Freezers of all three standard temperature levels (near 0°C, -20°C, and -80°C) must be available as a standard in biological laboratories in each containment area to avoid unnecessary pass-in/pass-out procedures. Since containment areas are restricted in space, this can lead to temperature disturbances due to the great heat dissipated by 80°C deep-freezers. In Europe, some suppliers offer freezers and other laboratory equipment (like ultracentrifuges) with a built in water cooling unit to limit additional heat dissipation to the laboratory space. Consequently, the choice of liquid-media cooled freezers is highly recommended.

Biosafety Cabinets—Some suppliers offer Class II (B1 or B2) biosafety cabinets (BSC) with a built-in water-operated cooling unit (SCAN AG, www.skan.ch) to reduce heat dissipation to the lab space. For safety reasons, the cooling systems should be operated in closed circles. Class-II-B2 BSCs are also suitable for handling small amounts of toxic chemicals and radioactive compounds; they are hard-ducted to the exhaust air system and do not redirect exhaust air to the lab space. Additionally, the ducts are equipped with mechanical flow indicator flaps for easy flow control. Because air removed from the laboratory will be fully substituted by the supply of fresh air,

air handling and pressure control systems must be designed to handle huge differences in the net air exchange rate, which is dependent on the number of currently active BSCs. Volume changes in the net air exchange rate may not have any influence on the pressure cascade arrangement of the individual laboratories.

Autoclave (in the BSL-3 Laboratory)—Some manufacturers offer autoclave models that do not require a vacuum operation mode, but meet high pathogen specification (3.4 bar, 134°C). The decision for a particular type must be based upon the kind of waste and material to be sterilised. If porous materials, such as HEPA filters, are to be treated, the penetration depth of the steam or steam-air mixture must be sufficient to permeate the material completely. Preferably, the main autoclave should be a pass-through model (Figure 6) to simplify waste logistics as far as possible. Another (maybe smaller) autoclave should be available in the laboratory as a backup autoclave in case of the other autoclave being inspected or out of order. It does not necessarily need to be a pass-through model.

Further specifications that must apply:

- All programs must be designed in a way to ensure that after a non-successful termination of a sterilisation cycle, the contents can only be discharged into the BSL-3 laboratory
- In case of an emergency, it is only possible to unload the autoclave into the BSL-3 laboratory.
- Some pass-through autoclaves can be programmed as a pass-in airlock (without thermal cycle on the way in) for the transfer of living animals and test specimens into the containment. Even in this particular case, an opening of the autoclave from outside may only be possible after termination of an additional sterilisation cycle.
- If a model with a vacuum operation mode is chosen, the exhaust air from the vacuum pump has to pass through an high efficiency particulate air (HEPA) filter, or an equivalent inactivation device (air incineration unit).
- All condensates have to be sterilised, ideally by inactivation simultaneously with the main process, or by in-line

Figure 6

Water barrier in the airlock.



sterilisation.

- In case of an emergency, all media supplies (air, steam, water) must be disengageable from outside the laboratory. For all supply lines, a fail-safe design has to be chosen.

Waste-Water Treatment—As one of the major material flow paths, the waste-water treatment line is by definition one of the most important containment interfaces at the end of a research unit, or an animal or production safety facility. All penetration points of the containment unit are sensitive to potential damage and represent “weak spots” in the containment shell. Particular attention must be paid to the waste-water inactivation path, when the decontamination device cannot be integrated into the containment. In this case, the decontamination device must be seen as an extension of the containment shell and be subjected to the same safety standards as the containment unit itself. Consequently, adequate safety measures for the waste-water-inactivation compartment must be developed during risk assessment and integrated into the safety concept.

Waste water can be biologically inactivated (sterilisation) by chemical or thermal means. This can be done “*in situ*” with the use of some smaller “flow-through autoclaves,” which are leading to fast and convenient sterilisation, and are also causing higher costs, if more than one device is needed. Alternatively, a pseudo-continuous “batch-flow-through” process can be applied. This approach combines the security of a batch process with the efficiency of a flow-through system. The main advantage lies in the more consequent implementation of redundancy and control principles as compared to a flow-through system, but with much greater performance compared to a pure batch system. In the end, waste water can be sterilised using central inactivation units. Such systems must be monitored constantly for safety reasons. A central facility might be energetically less expensive and economically more convenient.

Organisational Measures

In general, biosafety concepts enforce the effective interplay of technical and organisational safety measures. They are available on the international market and in different qualities. However, none of these will come into operation if they are not adapted to the local technical standards and organisational environment in a specific and individual manner. The reasons for this are outlined in the following sections.

Access Control—As emphasised earlier, access to the laboratories must be controlled using electronic personal identification and locking equipment (badge system).

Airlock—An airlock with interlocking doors is highly advisable since, from the technical point of view, the containment principle cannot otherwise be kept intact, if circumstances are troublesome. In some cases, interlocking doors may not be economically affordable; in this case, additional organizational measures will have to be

put into force. Nevertheless, practise has shown that it is very easy to overrun the airlock principle by keeping the doors open while passing in and out without hindrance. Although the air pressure gradients may remain intact, laboratory staff can become vectors of pathogenic and genetically modified organisms. This highlights the necessity for professional staff training and calls for a deeper understanding of containment principles.

Occupational Health and Safety

It is important to note that before commissioning of the laboratory, a site-specific occupational health and safety plan (OHS plan) must be formulated as a draft. The draft version allows for the testing of the laboratory and all procedures on an operational scale and under normal working conditions. Experiences documented during the first few months of operation will flow into the final version.

The OHS plan must address at least the following topics:

- Biosafety officer and safety committee (who are they and what are their responsibilities?)
- Risk assessment
- Education and training programmes
- Safety checklists
- Good Laboratory Practice (GLP), if required
- Pest control program
- Handling of sharp and other “risky” material
- Use of personal protective equipment including gloves, overalls, masks or respirators and goggles
- Disinfection and inactivation procedures and schedules
- Operational procedures and manuals for controlled access
- Safety rules for support staff (standard operational procedures)
- Contingency plan

Animal Welfare

Since the conditions within the rooms and cages fundamentally influence the well-being of animals, room construction and implementation of husbandry should strictly follow the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) guidelines. Some of the most important physical characteristics are listed below:

- Impervious walls and floors
- Self-closing doors and rodent barriers
- Lowest negative pressure (-60 ± 10 Pa) in BSL-3
- Regulated room temperature ($22 \pm 2^\circ\text{C}$)
- Maintenance of constant humidity ($55 \pm 10\%$ r.h.)
- Appropriate lighting (400 lux)
- 12/12 day and night cycle of lighting
- Minimized noise levels (<60 dBA)
- Balanced ventilation
- No air recirculation

- Appropriate air-exchange rate (12 air exchanges per hour)
- Strobe alarm lights for emergency situations
- Full capacity of heating, ventilation, air conditioning (HVAC) system running all time without turn-downs at night.

Safety and Security

Fire Protection—As a part of a multi-tenant complex, the fire-fighting strategy for BSL-3 laboratories must be seamlessly integrated into the fire-fighting strategy for the whole building. Knowledge of municipal fire authorities' policy is a prerequisite for the development of a gapless fire alarm and fire fighting strategies. The installation of a high-pressure sprinkling system reduces the amount of water used in case of a fire. Alternatively, a gas suppression system can minimise, or even avoid, water use.

Water used for fire-fighting in a BSL-3 containment must be held back (Figure 6). The floors within the containment shell can be shaped as shallow watertight trays to withhold any water for chemical or thermal treatment before final disposal. Alternatively, floor drains may be provided with a separate effluent collecting system.

Smoke Detection—As it is important to detect fire in the early stage of spreading, a state-of-the-art smoke and fire detection is the most efficient and effective way of fire protection. The system is part of the integral alarm cascade system concept.

Alarms and Coverage of Malfunction Events—Automatic fire alarm systems with direct alert of the fire departments control centre and redundant design are standard nowadays. In some countries and areas, mixed alarm systems, with segments which meet older, but not current, standards are still in operation. It is the duty of the safety experts in charge to ensure that the reliability of the alarm system is in good proportion to expected risks. In addition, it might be advisable to amend the "Building Management System" (BMS) with an automatic notification system that alerts the responsible scientists and service staff in case of any malfunction. This can be done with a short message service (SMS) notification system, which is highly independent, since it can rely on the battery backup of the BMS components. Such a system would even work in the case of complete power failure.

Containment of Fire-Fighting Liquids—To prevent the release of a large amount of potentially contaminated liquids, fire-fighting water will be retained separately. The facility itself is designed as a sort of retention unit. For this purpose, the lower portions of walls (10-12 cm) are sealed. No floor drains are located in the retention area. Fire-fighting water, or larger spills, will be collected with self-aspirated pumps designated for biosafety clean-up purposes.

Power Supply—Airlock, freezers, IVCs, BSCs, the BMS and similar devices are of critical importance to laboratory safety and function. They must be functional

at any time. Consequently, electric power should be supplied by two independent power lines: One regular power supply as well as an emergency power generator. A monitoring system, equipped with a battery-operated backup device, provides additional reliability. An uninterrupted power supply (UPS) has to safeguard most critical installations in addition to the emergency power generator. The surveillance devices, in particular the BMS, should be backed up by an independent (battery) power supply to allow for a self-initialising restart procedure after a power failure. Generally, an optimal power-supply system is expected to exhibit some "self healing" characteristics. Energy-consuming installations like autoclaves only need monitoring since they will automatically restart after having returned to normal power.

Room Fumigation—Room fumigation is a standard requirement in international biosafety legislation. In its essence, it is quite an "old" requirement stemming from a time when (biosafety) research was in its very early stages. In practice, fumigation is a safety measure for exceptional rare events. In the current legal enforcement of biosafety ordinances, fumigation is often seen as an ultimate measure after a major accident. Nowadays, numerous experts tend to interpret room fumigation as a measure of minimising the risks of re-entering a hazard area, but not as a standard disinfection measure. The latter interpretation results from the numerous difficulties encountered in successfully validating all the parameters that influence the effectiveness of the fumigation process (agent concentration, agent consumption, penetration of porous materials, and accessibility of cavities).

Fumigation is feasible in single compartments such as filter stations or biosafety cabinets. In a single compartment, fumigation can work as a sanitation measure on a regular basis (prior to conducting maintenance procedures).

For room or chamber fumigation, floors, walls, and ceilings must have non-porous and non-absorbent properties. An epoxy coating is a standard minimum requirement for walls and ceilings. In addition, the room or chamber must be leak-tight, with all penetrations adequately sealed to prevent the escape of the fumigant.

Biohazard Warning Labels—Biohazard warning labels are a must at the entrance to the BSL-3 area and recommended for "critical" areas such as BSCs and other storage devices for contagious agents. Labels must be clearly visible and comprehensible to staff members. Pictorial elements must be in agreement with international standards.

Additional Measures in Animal Care Facilities

Many of the recommendations given in the following paragraphs as "have to have" are currently under discussion as requirements for the next revision of Swiss biosafety legislation. Since the client required the laboratory to be in accordance with Swiss legislation, these recommendations have been turned into requirements in the

concrete example (chapter 6).

Storage units for infected animals are recommended to be located in a separate area of the facility within the BSL-3 perimeter. Biohazard warning labels are recommended for “critical” areas such as solid waste storage. They must be placed next to entrance doors. Doors to animal care rooms “must” be lockable.

Solid Waste Management—Autoclave or incineration equipment “must” be present in the laboratory. Alkaline liquefaction units are smoke-free and environment-friendly as compared to small-scale incineration devices. Such a device is acceptable with respect to biosafety provided the process is validated.

Alternatively, carcasses might be transported as dangerous goods (class 6.2 according to IATA) to an accredited incineration facility. For transport, containers suitable for class 6.2 transports must be used and the carrier must hold permission for the transportation of dangerous goods.

A more pragmatic approach might be the freezing of infected carcasses in suitable containers inside the BSL-3 and subsequent batch decontamination of the frozen carcasses by autoclaving. This approach is currently under discussion with the Swiss legal authorities. Sterilized carcasses are allowed to be frozen outside the BSL-3 laboratory until transport to an external incinerator can be arranged. It is crucial; however, to standardise and validate the decontamination cycle of the autoclave beforehand by simultaneously using biological indicator tubes and physical test instruments.

Usage of Individually Ventilated Cages (IVCs)—Individually ventilated cages (IVC) are not capable of excluding potential cross-contamination and the release of airborne pathogens by one hundred percent. Thus, IVCs cannot replace personal protective equipment (PPE), or general safety measures when animals infected with airborne microorganisms are handled. Appropriate filter masks (or respirators) must be worn at all times inside the animal unit. To minimise cross-contamination, IVC models with one-way air supply are highly preferable.

Concrete Example: The Novartis Institute for Tropical Diseases (NITD)

To illustrate the reflections above, the construction of the Novartis Institute for Tropical Diseases (NITD) (www.nitd.novartis.com) in Singapore is described in the following sections. Many of the recommendations provided above as “have to have” are currently under discussion as requirements for the next revision of Swiss biosafety legislation. Since the client required the laboratory to be in accordance with Swiss legislation, these recommendations have been turned into requirements in the concrete example.

Figures 7a and b show floor plans of the BSL-3 laboratory in the 8th floor (Figure 7a), as well as the equip-

ment room for the waste water treatment in the floor below (Figure 7b).

Construction and Design of the Laboratory

Architectural Measures—We integrated the laboratory unit into a pre-existing building and applied the “shell-in-a-shell” principle (chapter 4.2.1) by building inner walls. The inner shell was built up as a lightweight construction with its own self-supporting framework. An internal ceiling was installed above the laboratory unit to create room for the energy and media supply lines. The inner ceiling is a walkable construction that allows for easy access to all relevant parts of the installation. We laid all lines, pipes and channels from above to avoid wall penetrations. This way, a correct and almost gas tight line penetrations could be accomplished.

This “open installation concept” allows for easy and safe access for inspection and maintenance for all components. The safety concept is based on the idea that the inner shell of the lab suite, as well as each individual inner room, is as tight as possible. All inner surfaces (floor, ceiling, walls) have to be as flawless and resistant to aerosol diffusion as possible. All window openings were tightly integrated into the walls using a secure double-pane construction.

We defined the space between the inner shell and the building as lower-level containment. These are:

- The false ceiling
- The observation gallery
- The service area of the autoclave; the vestibule. For these spaces, a minimum level of leakage must be attained to maintain negative pressure under all weather and wind conditions and in all states of operation. The discharged air coming from these rooms will also be emitted via HEPA filters. We arranged and tested all inner rooms (including filter station and waste-water sterilisation station) for and by creating a pressure cascade of individual levels.

Ventilation and Air-Conditioning—All laboratory compartments are individually ventilated. The specific air-conditioning parameters in a given room are based on key values, such as room volume, intended purpose of use, equipment properties, such as heat dissipation and permanent media supply lines. All data are defined and filed in room-specific specification data sheets. The room specification data also include factors such as airflow, relative pressure, temperature, humidity, and quality specifications.

To attain a nearly fail-safe high-availability air-conditioning system in the level-3 containment, a highly redundant air conditioning system with two independent parallel lines has been installed next to the building’s standard air supply. These two lines are powered by redundant power lines, run parallel and simultaneously,

Figure 7a

Floor plan of the BSL-3 laboratory level 8 including pressure cascade.

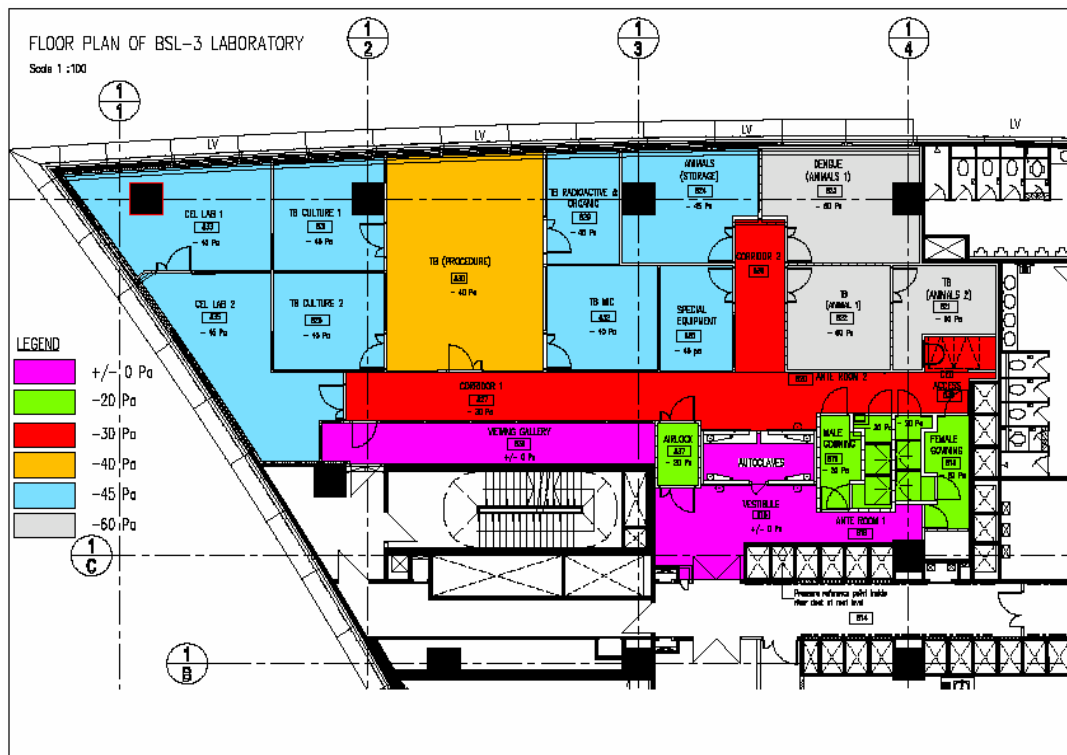
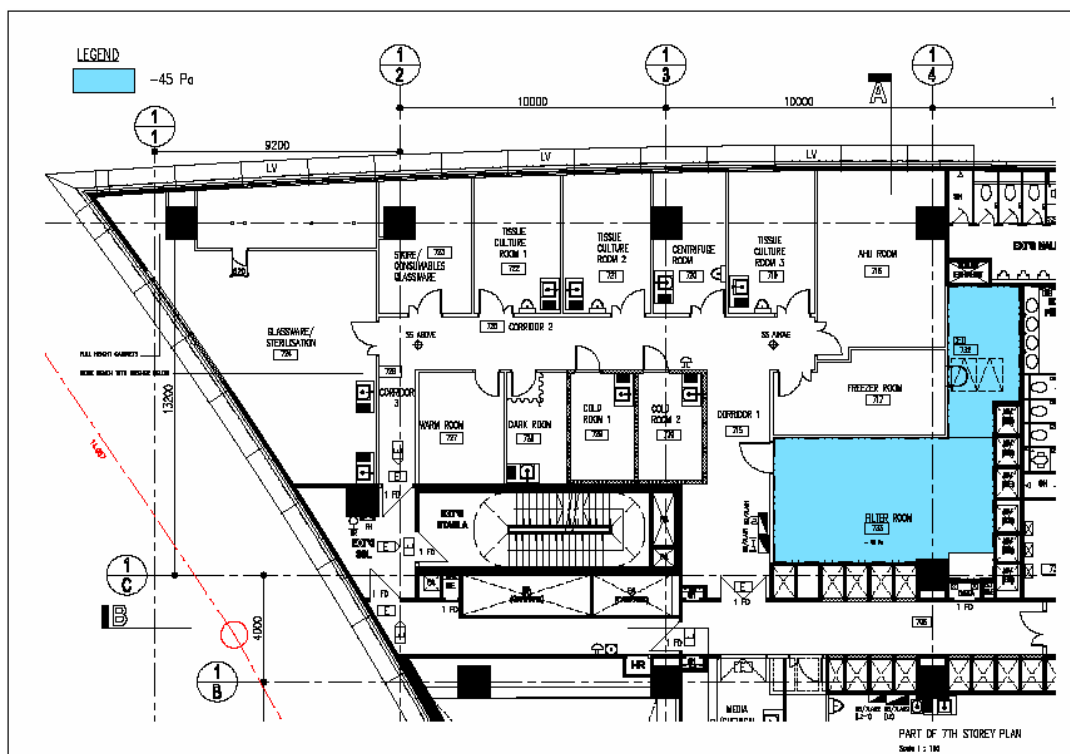


Figure 7b

Floor plan of level 7 including pressure cascade in the equipment room for the waste water treatment plant, which is part of the BSL-3 containment.



and are electronically cross-controlled.

The supply air intake is on the edge of the roof. Incoming air will be filtered three times (F6 and F9 as pre-filters and H14 [acc. to EN779 and EN1822]) and cooled in the HVAC system, before being delivered to the containment suite. In terms of biosafety, the international guidelines do not demand supply-air HEPA-filtration for BSL-3 laboratories; but taking the very humid and warm climate conditions of the location into account, the decision for supply-air HEPA-filtration was made to prevent any spreading of potential contaminants, such as fungal or bacterial spores.

The supply air runs through a common distribution channel using electronically controlled (power current frequency shift controller) ventilators. Their work is based on relative air-pressure reference values (VSD). The supply of each particular room is under the control of a “volume flow” control box that runs on a constant flow-reference basis. If necessary, the supply air can be heated before being distributed by the room diffusers. Each supply and exhaust air duct is equipped with gas-tight, powerless self-closing dampers, allowing for single-room isolation in case of an emergency, or for the purpose of fumigation.

The concept of individual pressure control in each room allows for setting up a differential pressure regime in the whole lab unit. The pressure-cascading scheme is based on the potential sources of cross-contamination, as well as on the risks posed by the use of pathogenic organisms. The lowest pressure levels are set in the animal storage rooms, the central HEPA-filter station and the wastewater treatment station. Air pressure, humidity, and temperature are under electronic monitoring in the most

important rooms.

Central Filter Station for Exhaust Air—Three individual filter stations (two of them always running simultaneously) comprise the central filter station. Each filter station consists of pre- and final (F7, H14, respectively) filter housings. “Safe-change” design was applied: Low leakage dampers can close off each filter station. Flanges may be connected to a formaldehyde generator in order to carry out effective fumigation prior to filter change. HEPA filters from the central filter station filter all exhaust air. The central NITD filter station is located in a maintenance room one floor below the lab unit. After filtration, exhaust air is directed to the corresponding exhaust fans on the roof. Each of the three independent filter lines provide 50% capacity to allow for 100% operational capacity even during shut down of one individual line for maintenance. The exhaust system is controlled by a constant-pressure system connected to a variable speed driven ventilator (so called VSD).

Sanitation—Cold- and warm-water supply lines are equipped with back flow-prevention valves to protect public supply lines in case of technical failure. Waste water from the BSL-3 rooms is collected one level below the lab unit and thermally inactivated in a flow-through process. Only after completion of an inactivation cycle is the waste water released into the public sewer system. Monitoring of all critical physical and chemical parameters guarantees compliance with legal requirements at any time. For biosafety reasons, the room containing the waste-water and HEPA-filter stations is part of the BSL-3 containment. Within the laboratory, lab staff and visitors have to wear full personal protective equipment (PPE). As a preventive

Figure 8

Two of the three HEPA-filter stations for filtration of the exhaust air.



measure against the potential release of aerosols, the waste water inactivation department was integrated into the pressure cascade of the containment shell. Under standard operation conditions, this room can only be entered through the safety laboratory and the appropriate safety rules must be followed.

Facility for Animal Experiments—Long-term experiments have to be performed in an environment, where each animal holding room is attached to a procedure room. In those rooms, animals are kept in individually ventilated cages (IVC) (www.biospectrumindia.com/content/Bioproduct/10508231.asp). Cages must be equipped with HEPA-filtered air, and air-pressure within the cages must be adjustable to negative or positive. All animal experiments have to be performed within bio-safety cabinets (BSC), which are also ventilated via HEPA filters. Using IVC cages and BSC prevents the staff from being infected. Short-term experiments have to be performed in a room equipped with an IVC rack and a BSC. The BSC must be placed far from the IVC rack. Animal food pellets, bedding, clean cages, and other consumables used in animal experiments have to be stored in a separate storeroom.

Continuous Effluent Decontaminator (CED)—The Steris CED system is an innovative waste-water decontamination system, designed to decontaminate and inactivate potentially infectious effluents in a flow-through process. The CED system consists of the following basic devices: collector tank, decontamination heat exchangers, circulation and discharge piping and control system. Decontamination of waste-water is achieved by heating water in the heat exchangers using saturated steam.

Construction

Monitoring—The setup process was attentively accompanied by the supervision team. Most of the critical technical equipment was set up first and tested in a pre-built mock-up room. Availability of materials, affordability and practicability were the most important decision-making factors. The knowledge, skills, and experience of staff members onsite are also always basic success factors.

As a key factor of communication the development of the construction was documented in step-by-step protocols, and deviations have been recorded by digital images. In order to bridge cultural gaps, the protocols were not only used to establish a documentation of construction, but were also helpful as a basis for discussion amongst staff members, the supervision team, or the authors, respectively.

Testing—We defined the testing schedule and procedures mainly following the WHO instructions. (WHO, 2004) The predominant tasks were supervision, controlling and advising during the qualification procedures. Topics, test parameters, and monitoring principles of the qualification procedures were primarily defined in accor-

dance with ISO 14644/4 (“Clean rooms and associated controlled environments—Part 4: Design, construction and start up”). To keep pace with a very tight construction schedule, testing procedures were sequentially conducted in coordination with the construction progress. The test sequences were carried out as following:

- Room integrity test
- Measurement of Air Handling Unit (AHU) capacity
- Supply air balancing
- Measurement of exhaust fan capacity
- Exhaust air balancing
- Water balancing
- Filter test
- AHU/fan/heater battery/pump performance test
- Control system test
- Measurement of temperature, relative humidity, and room pressure.

Parts of the qualification checks were already completed during the early stages of construction (tightness of media supply lines; tightness of the duct work, as well as the testing of the individual rooms). We have documented the results in a way to make them comparable to design specifications. The supervision team checked the results on the basis of periodic samples.

The most relevant technical guidelines during the qualification process were:

- ISO 14644/3 “Clean rooms and associated controlled environments—Part 3: Test methods” (filter-leak test, airflow measurements, pressure measurements),
- The European Standard prEN 13779 “Ventilation for non-residential buildings—Performance, requirements for ventilation and room-conditioning systems” (leak tightness of rooms and duct work systems), (a draft of the now valid EN 13779 standard)
- The British Guidelines.

Commissioning

As soon as all devices were available, they were setup and checked. The work was carried out under close supervision of the installation qualification team (Installation Qualification: IQ). Start-up sequences for all individual devices and systems were performed. All tests were carried out in the presence of representatives from the supplier/manufacturer to optimise and fine-tune all important operation parameters.

After the successful completion of the setup, we continued the commissioning process following the Operational Qualification (OQ) and Functional Qualification (FQ) process guidelines, as they are provided by the ISO standards. Beforehand, each test procedure was defined in coordination with the installers/manufacturers. We always included the following steps and recordings:

- System definition and description of function
- Test goal, physical target parameters and procedures
- Definition of important preconditions
- Definition of expected results

- Set-up of test conditions and specifications (How to start a test? Who has to be informed? Is the test sequence in correct order? What are the expected measurement values?)
- Required resources (staff, working time, instruments, documents, etc.)
- Analysis of test results (Was the test successful? Are the results in compliance with expectations and specifications?)
- Remarks, explanation, information
- Date with signature of the responsible engineers

Before handing over the whole installation to the owner, we ran a final test series on all external functions, effects and communication signals. This final test series was designed as an integral test to simulate “status of operation” realistically and to check for emergency situations such as failure of critical containment components, fire or power failure. The tests also included the switch over between standard power and emergency power conditions and vice versa.

Documentation

A further key task was to ensure correct and complete documentation. Construction and function-relevant data (including calibration of all devices) as well as procedures for operation and maintenance had to be documented completely and flawlessly. In accordance with ISO 14644/4 (Clean rooms and associated controlled environments—Part 4: Design, construction and “start up”) the overview of the whole system should be reflected by the documentation in a clear and easy to understand manner. Documentation must be accessible at all times to all responsible co-workers involved in start-up procedures, qualification, handing over and maintenance. Even more important, the documentation had to clearly and unambiguously reflect each staff member’s individual role and responsibility. The documents provided had to include the following details:

- Scheme drawings and description of function, schemes of pressure cascading and specification of hygiene classes
- Specification of operation parameters in accordance with the contracted user requirements, as well as the results of all relevant tests including all test logs
- Drawings, wiring schemes, Process and Instrument Diagrams (P&ID) schemes, and operation schemes as hard copies as well as electronic data and all amending requirements and agreements referring to the building contracts
- Spare-part lists with links and hints for spare-part supply and maintenance
- Documentation about manufacturers and suppliers as well as supply resources
- Comprehensible operation procedures for installations and devices, including the following details:
 - Start-up and shut-down sequence under standard

- conditions and exceptional conditions, respectively
 - Type and periodicity of routine tests and measurements
 - Description of test and measurement procedures
 - Required measures and warnings in case of emergency
 - Change management procedures and procedures after deviation from requirements and specifications.

Instructions for Maintenance

For an ordinary standard operation of the safety laboratory, specific test and maintenance procedures have to be defined in the maintenance procedures (M-SOPs) and performed on a regular basis. These maintenance procedures have to be periodically conducted in a predefined order, as documented in the maintenance plan. Additionally, the maintenance procedures can be started on an event basis if deviations from the standard operation parameters are monitored.

During room qualification, the standard operational parameters, such as pressure differences, airflow volume, air changes per hour, temperature, humidity, filter-leakage rate, also including all tolerance ranges, have to be standardised. The related values always have to be used as a reference for all follow-up routine tests during maintenance and re-qualification. For document checks, the following aspects have been given special attention in agreement with the ISO14644/4 (Clean rooms and associated controlled environments—Part 4: Design, construction and “start up”):

- Definition of maintenance measures
- Definition of safety measures before maintenance or repair works
- Change management: definition of acceptable changes and standardised change procedures;
- Checking requirements and exchange of spare parts as well as the rules for the disposal of spare parts, such as belts, and filters
- Definition of all actions, procedures and checks that are required for the standard operation status
- Consideration of all user-specific and legal requirements or ordinances to be obeyed.

All maintenance work under standard operation conditions are logged in maintenance reports. These reports have to include the following:

- Definition of individual maintenance procedures
- Naming and authorisation of maintenance-staff members
- Date, time, and description of the maintenance job done
- List of used spare parts
- Report log about the condition of the parts of installation under maintenance before and after a maintenance job.

From the time of the start-up sequence, some of the owner’s responsible co-workers were involved in the quali-

fication process. This way, efficient information transfer to the owner is guaranteed.

Certification

Apart from having to comply with Novartis' own internal biosafety requirements the BSL-3 unit described here had also to be certified by Singapore's authorities. The latter was completed during a transition period when biomedical legislation was under refinement and ratification by the Parliament. Some legislation had reference to existing biological safety and security; still, it was neither complete nor sufficiently comprehensive to regulate all aspects in terms of construction, commissioning and operation. Nevertheless, the authorities recognised the importance of the matter and anticipated some important impending legislation. The resulting paragraphs detail the key approvals/licenses that were secured for this particular BSL-3 unit. In addition, being incorporated within a BSL-3 facility meant that it was also necessary to obtain approval for animal research.

Approval as a "Certified Facility" to Work with Risk-Group-3 Pathogens—This particular approval was obtained from the Singapore Ministry of Health (MOH). The MOH is the key administrator of the Biological Agents and Toxins Act (BATA), released in January 2006. BATA is now the most important piece of legislation governing the approval of BSL-3 units as described here. In Singapore, BATA regulates the possession, use, import, transfer, and transportation of biological agents and toxins that are known to be hazardous to human health. Although BATA was not in force at the time we applied for certification, the MOH leant on BATA guidelines as closely as possible. MOH also adopted the *Laboratory Biosafety Manual, 3rd Edition*, published by the World Health Organisation (WHO) as the national guidelines for biosafety compliance.

For our certification process, the MOH adopted a "stand-back" approach: The MOH maintained its public accountability by creating a legal framework with basic requirements. It is the responsibility of the NITD; however, to guarantee safe operation of the BSL-3 laboratory. The certification audit was carried out by a NITD-appointed and MOH-approved auditor. The audit was comprised of a three-day program including interviews with responsible NITD staff members, confirmation of technical aspects, verification of documents and filing system, commissioning, as well as site inspections. On the final day, the certification audit culminated in a fruitful meeting with MOH officials where the key findings of the audit were presented.

Import Permit for Infectious Agents—Section 41 of the Infectious Diseases Act administered by the MOH states that "no person shall, without the prior written permission of the Director of Medical Services import, or bring, or cause to be imported, or brought into Singapore, any disease causing organism, or any agent of disease capa-

ble of transmitting a disease." NITD successfully obtained such a permit for *Mycobacterium tuberculosis* as it was tied to the certification (see "Fire Protection" above). The first imported *M. tuberculosis* arrived safely in June 2005.

License to Possess Veterinary Biologics (for Research and Development Purposes)—As NITD had also planned to work with Flaviviruses, such as West Nile Fever, Yellow Fever and Japanese Encephalitis, another license was required from the Agro-Food and Veterinary Authority (AVA). A one-year renewable license was issued to NITD upon successful completion of a site audit by AVA inspectors.

License under the Animals & Birds Act; Rules for Animals and Birds—Singapore's rules for the administration and management of animal research facilities were not released before the end of 2004. Until then, the Agro-Food and Veterinary Authority (AVA) requested NITD to comply with the Guidelines on the Care and Use of Animals for Scientific Purposes developed by the National Advisory Committee for Laboratory Animal Research (NACLAR). These guidelines were in accordance with widely accepted scientific, ethical and legal principles and constituted the best practice in the use and care of test animals. After a successful site audit in the ABSL-3 facility by AVA animal-welfare inspectors, NITD was issued a one-year license.

Gazetting as a "Protected Place" under the Protected Areas and Protected Places Act—The Ministry of Home Affairs and Singapore Police Force require any facility working with certain agents found in the proposed First Schedule, Part 2 of BATA (see above), to be gazetted as a "Protected Place." "Gazetting" is the Singaporean synonym for publishing. Such agents are defined as risk-group-3 biological agents, which can cause serious diseases, but also have the potential for being abused for the development of biological weapons. Future work with the Yellow Fever virus proposed by NITD fell into this category. Based on a security audit by the Ministry's officials and subsequent enhancement of security installations and measures, approval was given to NITD.

Summary

The handling of pathogenic and/or genetically modified organisms requires high levels of safety standards, even more so when handling genetically modified pathogens. As a consequence of the high developmental pace of modern biotechnology, these safety standards have to be continuously adapted.

The project manager, Health and Safety Executive (HSE) and biosafety officers had to take a key role in the knowledge transfer to staff and in the start up process. These persons were also involved in the detail planning as well as in the definition of technical and organisational interfaces. Nowadays, they are equipped to monitor every deviation from the reference specifications for the stan-

dard operation. This way, they are ready to initiate the appropriate measures and necessary actions at any time.

Through the nature of legislation development with certain feedback processes, such as in public opinion forming, jurisdiction and standardization, there is always a natural delay between the prevailing demands of modern biotechnology and the standards reflected in the laws and ordinances of modern biosafety legislation. This publication describes the build-up, implementation, commissioning and certification of a BSL-3 laboratory in an environment where biosafety legislation is still under development. The chosen approach of setting up a safety laboratory integrates modern risk assessment strategies with:

1. The requirements of various international biosafety guidelines
2. The development of new safety measures and certification procedures according to international guidelines
3. A proactively open communication strategy to the public and local legal authorities.

Besides that, the project had to fit into a very narrow time schedule because of its integration in a WHO project.

This publication shall contribute to the rapid development of modern safety legislation and serve as a preliminary template for future processes in the South Asia Pacific area.

Conclusions

The installation of a BSL-3 unit in a drug discovery environment within a mixed multi-user and multi-tenant estate presented the supervision team with a complex starting position. Operational safety and security, as well as user requirements and public perception were subject to conflicting demands. Controversial issues had to be identified and tackled at an early stage. Subsequently, input from numerous branches, such as management, financial support, lab design and engineering, pathogen safety and security and, finally, legal compliance and registration services had to be assessed by the supervision team.

All conclusions were merged into an overall implementation concept and formed the basis of various innovative project management strategies.

Time restrictions and limitations in the availability of skilled craftsmen, as well as materials in the Singaporean market, made it necessary to transform some European technical specifications into objective-oriented ones. In this situation, instant “out-of-the-box” solutions were in high demand.

The ultimate prerequisites to start the commissioning process at the end of the installation phase were purposeful communication strategies, gapless documentation, and practical staff training. The commissioning process involved instantaneous “on-the-job training” for maintenance and service staff. The quality of documentation and the trainings turned out to be indispensable key factors in

the efficient realisation of the certification procedure.

NITD, represented by the supervision team, was committed to build a safe level 3 biomedical research facility compliant with international standards. The development process was the result of a close cooperation between government authorities and industry. This cooperation marked a significant step towards a modern and sustainable biomedical research community in Singapore.

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Mobile Biosafety Level-4 Autopsy Facility—An Innovative Solution

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Abstract

Recent threats of bioterrorism, outbreaks of previously unknown infectious diseases such as Severe Acute Respiratory Syndrome (SARS) and the reemergence of diseases like the Avian Influenza are very real and have caused serious concerns not only for the world-at-large, but also for many authorities. This is an even greater concern for the forensic community as they are generally ill-equipped to deal with highly infectious pathogens due to chronic under funding and administrative constraints. The cost for building a Biosafety Level 4 (BSL-4) facility is exorbitant; such a facility is also very expensive to operate and maintain. Given the state of funding for most Forensic Centers and Medical Examiner Facilities in the world, having a high containment BSL-4 facility just to carry out autopsy work is highly unlikely.

In the course of dealing with the SARS outbreak in Singapore in 2003, the Centre for Forensic Medicine (CFM) of the Health Sciences Authority, together with its strategic partner, Acre Engineering, developed an innovative solution that would meet the requirements set out for a BSL-4 Mobile Autopsy Facility. This was completed at a fraction of the cost and in less than half the time spent building such a facility de novo. This paper therefore sets out to present an innovative solution to meet the need for an autopsy facility equipped to BSL-4 standards that can be mobilized and deployed at short notice to conduct autopsies on highly infectious cases at distant locations. In particular, it addresses the engineering and facilities components of the solution.

Background

Presently, very few nations are equipped with standard BSL-3 or BSL-4 autopsy facilities as such units are expensive to build, operate and maintain correctly. These existing contingency facilities are normally housed in permanent or semi-permanent structures and cannot be moved to distant locations to deal with emergencies involving BSL-3 or BSL-4 hazards. While “portable morgues” in the form of palletized mortuary supply systems organized by the U.S. federal government department under the Disaster Mortuary Operational Response Team (DMORT) are available, they have no capacity for biosafety stringency, or for microbiologic diagnosis (Nolte, 2003). The lack of a mobile autopsy facility poses a very real problem in that bodies infected, or suspected of being infected with RG-3 and RG-4 agents, cannot be autopsied in remote locations where such practice is most required. Nolte, Taylor, & Richmond (2001), addressed this problem when they were evaluating the status of Autopsy Facilities in the United States in their study of “Autopsy Biosafety” in 2001. They recommended that “a mobile containment autopsy facility constructed to operate at Biosafety levels 3 or 4 might be useful in providing autopsy support to jurisdictions with inadequate facilities when they are confronted with contagious or toxic cases.”

At the height of the SARS outbreak in Singapore in 2003, the need for developing a solution that would meet the requirements set out for a BSL-3 or 4 mobile autopsy facility became very apparent and pressing. The challenge was to meet the need for providing appropriate facilities