Commissioning of High-containment Laboratories: Bernhard Nocht Institute of Tropical Medicine, Hamburg, Germany

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Abstract

Currently, there are four BSL-4 facilities built or planned in Germany, all falling under the legal regulations for recombinant DNA. The authorization of these facilities is the responsibility of the local authorities of the Federal States.

This article reports on the commissioning of a high-containment facility in Hamburg expected to go into service in 2011. A matrix of about 100 critical containment components has been compiled by the local authority that led the inspectorate through the commissioning process. In cases where no acceptance criteria for certain tests were specified in neither the German nor the European regulations, the commissioning authority had to determine the international state-of-the-art standards which are often set by North American guidelines and regulations.

This article describes in detail some typical findings from the commissioning process: inter alia filter testing, room integrity tests, and validation of gaseous room decontamination.

Introduction

The number of high-containment laboratories is increasing worldwide, including in Germany. Currently, there are four new BSL-4 facilities built or planned in Germany: the laboratory of the Institute of Virology of Philipps University in Marburg, the laboratory of the Bernhard Nocht Institute for Tropical Medicine in Hamburg, the laboratory of the Friedrich-Loeffler-Institute in Riems, and the laboratory of the Robert Koch Institute in Berlin. The laboratory in Marburg has been in operation since 2008 and the laboratory in Hamburg is expected to go into service in 2011; however, the two others are still under construction.

All four facilities are approved according to the German Genetic Engineering Act (1993) because genetic manipulation by recombinant DNA techniques with high-risk group viruses is or will be performed. This Act and several ordinances form the legal framework for the handling of genetically modified organisms (GMOs) which are guided by European Directive 2009/41/EC on the Contained Use of Genetically Modified Micro-organisms (European Union, 2009). In Germany authorities for the approval of GMO facilities are located in the Federal States. For the City State of Hamburg, it is the Hamburg State Ministry for Urban Development and Environment, which is also the inspectorate for gene technology facilities falling under the scope of the German Genetic Engineering Act.

Outline of the Facility in Hamburg

Figure 1 shows the new extension of the Bernhard Nocht Institute ([www.bnitm.de/]) located in the inner city of Hamburg alongside the established Institute at the Elbe River. In addition to the BSL-4 facility, it will also contain three BSL-3 facilities and numerous laboratories at containment level 2. In the five-story building, two additional levels underground are reserved for the housing of small laboratory animals.

The BSL-4 facility itself consists of two laboratories of 77 m² and 39 m², respectively. Both are accessible through individual airlocks that operate independently. The larger one is additionally equipped with an animal room of 11 m² for the housing of small rodents in individually ventilated cages (IVCs).

The Hamburg laboratory is a suit-type laboratory. Entry and exit are via a three-chamber airlock. When leaving, the use of a chemical shower with peracetic acid is mandatory. Since the effluent decontamination plant is beneath the BSL-4 facility on the ground floor, part of the air-handling system is located three levels above. The inner shell of the containment is made of fully welded stainless steel plates, and supply lines are fitted in air-tight connections.

Commissioning Process

Laboratory/facility commissioning may be defined as the systematic review and documentation process signifying that specified laboratory structural components and system components have been installed, inspected, functionally tested, and verified to meet national or international standards, as appropriate (WHO, 2004). It is challenging to demonstrate that not only are single components working properly but also that the systems’ many components and different systems are also properly interacting in complex ways.

Since our inspectorate regards the commissioning process as the most important inspection of the facility, participation in onsite inspections during this commissioning process were deemed very important. To accomplish that in a structured manner, a matrix of critical con-
tainment components was assembled. Table 1 gives an overview of this matrix: Under each topic listed, a varying number of individual checks have to be performed. These items are primarily from requirements specified in the permit or in German legislation on the use of GMOs. In addition, existing checklists for the commissioning of high-containment laboratories served as a valuable source for information (Office of the Gene Technology Regulator, Australia, 2007; Public Health Agency of Canada, 2004).

The entire matrix consists of about 100 tests to be performed on behalf of the inspectorate for gene technology in Hamburg. In addition, the outline of the pressure decay test for BSL-4 laboratories, the content of the facility manual, the requirements for the training of personnel, the timing of recertification, and the nomination of responsible persons are further specified in the annexes of this matrix.

It should be noted that these tests are only part of the whole commissioning process because they focus on systems judged critical in their function to guarantee biosafety and biosecurity.

**Results of the Commissioning Process**

Some of the typical findings in the commissioning process are described in detail below.

**Figure 1**
The new building of the Bernhard Nocht Institute in Hamburg.

![Figure 1](image)

**Table 1**
Outline of the Acceptance Checklist for High-Containment Laboratories

<table>
<thead>
<tr>
<th>System to be Checked</th>
<th>No. of Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory equipment</td>
<td>23</td>
</tr>
<tr>
<td>Ventilation system</td>
<td>14</td>
</tr>
<tr>
<td>Air-tightness</td>
<td>3</td>
</tr>
<tr>
<td>HEPA-Filters</td>
<td>10</td>
</tr>
<tr>
<td>Autoclave, waste water treatment</td>
<td>13</td>
</tr>
<tr>
<td>Breathable air supply</td>
<td>4</td>
</tr>
<tr>
<td>Airlock, doors</td>
<td>10</td>
</tr>
<tr>
<td>Media supply and drains</td>
<td>8</td>
</tr>
<tr>
<td>Emergency power supply</td>
<td>12</td>
</tr>
<tr>
<td>Technical plat room</td>
<td>3</td>
</tr>
<tr>
<td>Fire-fighting system</td>
<td>4</td>
</tr>
<tr>
<td>Other</td>
<td>9</td>
</tr>
</tbody>
</table>
HEPA Filters in BSL-3 Laboratory Exhaust Air
Exhaust air in BSL-3 laboratories has to be treated by HEPA filters. The written requirements specify that filters of class H14 (DIN, 2009a) have to be used. These filters have an efficiency of 99.995% at the most penetrating particle size (MPPS) (DIN, 2009b). The blueprint shows HEPA filters of class H13 in the exhaust air line with a corresponding efficiency of 99.95%. Additionally, by looking at the filter-housing, it became apparent that the wrong filters had been installed (Figure 2). This test failed and the Institute has been asked to correct this.

In an additional test, HEPA filters were checked for the rate of air leaking between the frame of the HEPA filter and the filter housing. The corresponding German Norm (DIN, 1992) states that air leaking between the frame of the HEPA filter and the filter housing has to be smaller than 3 x 10⁻⁵ of the air-flow, measured at 2000 Pa. For the given filters and air-flow, this value should be smaller than 0.9 L/min. However, testing revealed a much better value of ~0.015 L/min.

In another test, filters were scanned for leaks by applying a test aerosol. As a consequence, one of the three filters tested had to be replaced.

Ventilation System
Supply and exhaust air must not interfere with biosafety cabinets. This could occur when the velocity of air from supply or exhaust registers of the ventilation system near biosafety cabinets disturbs their laminar and inward-directed air flow. The safe handling of specimens is no longer guaranteed under such conditions.

Figure 3 shows an air duct outlet just above a biosafety cabinet in the animal room of the BSL-4 facility.

This test failed and the Institute has been asked to find another position for the air exhaust.

Autoclave
Special emphasis has been put on the certification and validation of each BSL-4 laboratory’s double door autoclave. Amongst others, the sterilization cycle for the autoclaving of carcasses of small rodents has been validated by the use of thermocouple devices placed at various parts of the animal’s body. This validation step is important because carcasses are collected frozen prior to autoclaving. In the corresponding standard operating procedure (SOP) it has to be specified how to handle them so that the core of the body reaches a temperature of 121°C.

According to the German Genetic Engineering Ordinance, exhaust air extracted during the vacuum cycles of the sterilizing process has to be filtered twice by dual HEPA filters in series. Figure 4 shows that the two HEPA filters in series have been built in.

Waste Water Decontamination Plant
The room containing the waste water decontamination plant is located one level beneath the BSL-4 facility. A 450 L kill tank is heated to 134°C by injection of hot steam before the waste water is cooled down and discharged into the sewer system. It is connected to four large tanks collecting waste water from the two chemical decontamination showers, the various containment level 3 laboratories, and water originating from the fire extinguishing system in the event of a fire. As this room with its technical equipment supports the BSL-4 laboratories, it is part of the BSL-4 facility and must be sealable for

**Figure 2**
Wrong filters in exhaust air line.

**Figure 3**
Air outlet just above a biosafety cabinet.
gaseous decontamination, here with volatile hydrogen peroxide, if necessary.

To verify the overall air-tightness of this room, a blower door test was performed as part of the commissioning process. After the laboratory duct outlets were sealed and isolation dampers on supply and exhaust ducts were closed, a Minneapolis Blower Door (The Energy Conservatory, Minneapolis, MN) was installed in the frame of the entrance door. Air change rates at various differential pressures were determined both for positive and negative pressure. From these measurements the resulting $n_{50}$-value [$\text{h}^{-1}$] was calculated, indicating how many times per hour the whole volume of the room is exchanged at a differential pressure of 50 Pa. The smaller this value the better is the sealing of the room.

An initial $n_{50}$-value of 3 indicated that this room is not air-tight, and it was doubtful if it would be possible to perform a successful gaseous decontamination. Since gas leaking from the decontamination process must not hamper the surrounding work areas, the Institute was asked to improve the overall tightness of the room. One measure taken was the sealing of the stainless steel floor, which acts as both a base and a retention area for any fluid leaking from the tanks or pipes, against the walls. Additionally, the sealing of piping penetrating the envelope of the room was reinforced (Figure 5).

Subsequent blower door tests proved that the tightness of the room could be improved to an $n_{50}$-value of 1.6. The validation of the gaseous decontamination process itself will demonstrate whether the measures taken to reinforce this room have been sufficient to guarantee a safe and efficient decontamination process.

**Pressure Decay Test**

The German Genetic Engineering Ordinance specifies in its annex class 4 that “walls, ceilings and floors have to be tight against the outside world. All penetrations of service pipes and waste disposal lines have to be sealed.” Because this phrasing leaves too much room for interpretation, international guidelines for accepted limits of air-tightness of BSL-4 facilities were sought. It was decided to adopt the acceptance criterion for the integrity of containment level 4 laboratories specified in the U.S. Department of Agriculture’s *ARS Facilities Design Standards Manual* (USDA, 2002) and Canada’s *Laboratory Biosafety Guidelines* (Public Health Canada, 2004). In these the rate of air leakage is measured by a pressure decay test where the initial pressure of 500 Pa should not exceed a pressure drop to 250 Pa in 20 minutes. Test conditions must be conducted under generally stable conditions of outside wind, temperature, barometric pressure, and humidity.

In Hamburg this test was applied in both positive and negative pressurization. In each test once the laboratory’s inner doors and outer doors were opened giving rise to eight individual tests for the two main laboratories. By doing so it was possible to check the air-tightness of both doors of the chemical showers, the

**Figure 4**

Two successive HEPA-filters in exhaust air line of the autoclave (two filter housings equipped with manometers).
double door autoclaves, and the decontamination chambers for the transfer of materials.

To achieve the acceptance criterion for air-tightness, many corrections had to be accomplished, especially in the sealing of the piping through the containment; this was surprising not only for the user but also for the construction team.

**Validation of Gaseous Decontamination**

Gaseous decontamination of high-containment laboratories can become necessary due to several circumstances, including after a large spill or major accident, before maintenance work starts, with the removal of large equipment, or with the change of HEPA filters. In the new building of the Bernhard Nocht Institute, gaseous decontamination will be achieved by vaporization of hydrogen peroxide. To facilitate this, all high-containment laboratories at levels 3 and 4 are equipped with nozzles for the adaptation of gas generators and piping for the internal distribution of the gas within the room.

Because room sizes differ significantly, a validation of gaseous decontamination has to be done for every room. Spores of *Geobacillus stearothermophilus* as biological indicators were distributed in each room, mainly at spots judged to be most difficult to reach by the decontaminant. By varying the process time and concentration of the decontaminant, the most efficient conditions were determined.

During these validation steps, the spaces surrounding the laboratory were scanned for gas leaks by using a hydrogen peroxide gas detector (Dräger, Pittsburgh, PA) to ensure that the 0.5 ml/m³ H₂O₂ limits for worker protection are not exceeded. In one of these tests, the decontamination process had to be aborted due to the detection of gas leaking from the ventilation system in amounts greater than the upper limit of the detector of 5 ml/m³. As shown in Figure 6, taped connections for air ducts were identified as the cause of this finding. Although taped connections of air ducts are widely used in the construction of ventilation systems, they are not appropriate for high-containment laboratories.

**Biosecurity**

During the commissioning process of the new maximum containment laboratories at the Bernhard Nocht Institute, special emphasis had to be put on the issue of biosecurity. One way to do so is to restrict and document access to the organisms handled in the lab, notably various strains of viruses of risk group 4 or full-length clones of certain viruses in *E. coli*, by pointing video surveillance cameras toward the locked freezers in which the virus stocks are stored. To achieve this, the consent for the facility specifies that the image stream from the surveillance cameras pointed at the freezers containing the reference stocks must be recorded and stored for at least 2 weeks. When checking this, it was noticed that recording time covered only the last 5 days. So this test failed and the Institute has been asked for corrective action.

**Conclusions**

The commissioning of BSL-3 and -4 laboratories is an extremely time-consuming task. The commissioning authority has to focus its attention on a selection of containment measures considered most critical for the proper functioning of the facility.

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**Figure 5**
Inappropriate sealing of pipes against the wall.

**Figure 6**
Taped connections of air ducts.
By participating in the commissioning process of the Bernhard Nocht Institute of Tropical Medicine, the authorizing agency obtained thorough insight into the construction, function, and interdependencies of technical devices. This thorough understanding of the technique that supports the operation of such a complex facility will be invaluable during inspection and recommissioning in the future.

The fact that basic requirements for the construction and properties of high-containment laboratories, such as the degree of air-tightness, are not specified in the German Genetic Engineering Act and its Ordinances or in technical guidelines was very disadvantageous to the process. From the outset, the acceptance criterion for air-tightness should be known by all participating parties when planning the construction of a high-containment laboratory. These participating parties should include at a minimum the user, the architects, and the licensing authority. Reaching a high degree of air-tightness is a challenging task from the materials used to build the envelope, to the engineering technologies to handle these materials, and to the multiple interdependencies of installations inside the facility or passing through the envelope of the laboratory. If the ultimate goal to achieve a certain degree of air-tightness is not clear to all relevant parties, it becomes impossible or at least very expensive and time-consuming to reinforce the containment so it reaches the accepted criterion limits. According to the German Genetic Engineering Act, the state-of-the-art in science and technology has to be considered. U.S. and Canadian guidelines, the international literature, and personal contacts operating BSL-4 facilities in Winnipeg (Canada), Lyon (France), and Stockholm (Sweden) served as resources to find an appropriate limit for air-tightness for the new laboratories in Hamburg.

This article has presented a matrix of the ~100 critical containment components compiled by the authorizing authority. These served as a checklist for use throughout the commissioning process. This matrix was also very much appreciated by the construction team. An updated version of this matrix was made available after each on-site visit by the commissioning team. By doing so the current status of the whole process was transparent at all times.

Acknowledgments

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References


