

Safety Instruction Manual

July 11, 2000

****** Laboratory**

XXX Department, Faculty of YYY, ZZZ University

Research Project ID: 2001B0000 -np

Research Project: Crystallographic structure analysis of vaccinia virus particles

Beam line No.: BL01XU

Samples

1. Vaccinia virus
 - (1) Infectious for humans: Yes.
 - (2) Disease symptoms: Pathogenicity has been considerably reduced. Inoculation causes a benign skin lesion, although this is typically not contagious. However, a defective immune system might trigger serious symptoms.
 - (3) Infectious for animals: Infectious for vertebrata such as cows, rabbits, and mice.
 - (4) Remarks: Crystallized samples lack infectiousness for humans and animals.

1) Structure of the experiment team and reporting structure

- Structure of the experiment team

Affiliation: **** Laboratory, XXX Department, Faculty of YYY, ZZZ University

Principal investigator of biology experiment: Professor xxx, Years of experience in biology experiments: 20 years

Associate principal investigator of biology experiment: Assistant Professor yyy, Years of experience in biology experiments: 8 years

Experiment conducted by: AA BBB (student), Years of experience in biology experiments: 3 years

CC DDD (student), Years of experience in biology experiments: 3 years

- Reporting structure

The reporting structure is shown in Appendix 1.

2) Entering and leaving the Lab where classified samples are handled

An area of restricted entry should be provided in front of the Lab hatch of BL01XU where virus samples are handled. Entering and leaving the restricted area should be recorded in a log book. The log book should record the date, name, time, purpose of entry, and time of leaving.

Special lab gown and footwear should be worn whenever virus samples are brought in. Personnel should not leave the restricted area while wearing such clothing and footwear. Hands and fingers should be sterilized using 75% ethanol after handling the

samples.

3) Preventing contamination of the body and clothes when handling specific biological samples, and medications for preventing infections

Goggles, masks, and rubber gloves should be worn during an experiment in order to prevent contamination of the body and clothes. Glutaraldehyde and 75% ethanol should be immediately available during an experiment in order to avoid contamination.

Vaccinations should be provided as necessary for a particular experimental operation in order to prevent viral infections.

4) Operation of experimental facilities or local confinement facilities that may be contaminated by a specific biological sample

Install a safety cabinet inside the hatch of BL01XU to handle virus samples. The hatch door should be kept closed and a shutter operation key carried when handling a virus inside the lab hatch. Hatch exhaust equipment should not be operated without a filter.

The inside of the safety cabinet should always be clean and tidy. The floor around the safety cabinet should be kept free of objects as much as possible in order to prevent the spread of contamination. The floor should be covered with a sheet as necessary.

Appropriate measures should be taken to prevent small animals from entering the experimental hatch.

Strictly adhere to other precautions that are prescribed by the principal investigator.

5) Storage of specific biological samples (Storage facilities, locking method, and packaging while in storage)

Virus samples should be kept in the locked refrigerator or freezer dedicated to virus sample storage in the restricted area in front of the lab hatch. The refrigerator and freezer should be marked with signage as stipulated in Clause 31 of the *Regulations on Biosafety at SPring-8*, and these should not be used for any other purpose.

The keys to the refrigerator and freezer should be kept by the principal investigator.

Virus samples should be placed in crystal form in a glass capillary 0.2 to 1.5 mm in diameter and 0.01 mm in wall thickness. Both ends of the capillary should be sealed with dental wax or vacuum compound, and each capillary stored in a separate sample storage bottle. The capillary should be fixed to the lid of the sample storage bottle using compound. Several sample storage bottles should be packed in a tight box, and the tight boxes should be placed in a sealed stainless steel tank for transportation.

6) Disinfection and sterilization equipment to be available at all times - types, purposes, number, and storage locations

Types	Purpose	Number	Location
Autoclave	High-pressure sterilization	1	Lab hatch*
75% alcohol	Sterilization	2	Safety cabinet
Glutaraldehyde	Sterilization	2	Safety cabinet and Lab hatch entrance

(See Appendix 2)

7) Precautions for experiments involving specific biological samples

Verify that the glass capillary is not damaged when you remove a sample bottle from the tight box.

Virus crystals should remain in a glass capillary to be mounted on a goniometer. Sample position and diffraction intensity data collection should be adjusted remotely

using a computer inside the work control area. Except while data is collected, specific biological samples should be kept in a refrigerator or a freezer with signage stating that the specified biological samples are in storage.

Glass capillaries should not be removed from the transportation container outside the safety cabinet.

8) Handling log for specific biological samples

Whenever a biological sample is handled, the name of the person handling it and the date, time, location, and purpose should be recorded.

9) Disinfection, sterilization and disposal of specific biological sample experiment apparatuses, culture media, chemicals, animals, etc.

Items that may come in contact with virus samples during an experiment, including the following, should be brought back to the University after sterilization with high-pressure steam.

Sample holders used during sample transportation

Tweezers to handle samples

Culture media and chemicals also should be sterilized using high-pressure steam and brought back to the University.

Personal protective equipment, including goggles, masks and arm covers, should be placed in a plastic bag and brought back to the University.

10) Safety education and training for experiment personnel and records thereof.

The principal investigator of a biological experiment is responsible for conducting education and training regarding experimental procedures and safety measures, including the properties and handling of a vaccine virus and the handling of glass capillaries. The education and training should cover *Regulations on Biosafety at SPring-8*, *Detailed Regulations on Biosafety at SPring-8*, and *Criteria for Laboratory Facilities Where Samples are Handled and for Safety Work*.

Whenever education and training is conducted, the date, time, content, name of instructor, and names of students should be recorded.

11) Medical examination of experiment personnel

Personnel engaging in an experiment (including biological experiment principal investigator) should undergo a medical examination prior to handling a sample and another medical examination within one year from the start of handling the sample at his or her University. The results of the examinations should be used to determine whether or not a problem exists affecting the continuance of the experiment, and the decision should be communicated to the head of the Safety Office.

12) Inspection of BSL-2 Lab (Frequency of inspection of items such as filters in the safety cabinet)

Inspect the exhaust filter for clogging and the safety cabinet for any issues before and after the start of a research project. Record in the log the inspector's name and the inspection date, time, inspected items, and the results.

13) Restoration of lab after an experiment and record-keeping

After an experiment, disinfect with glutaraldehyde and 75% alcohol the locations where infectious samples were used. Items such as the safety cabinet should be returned to the original state. Inspect the exhaust filter for clogging and other issues. If an issue is found, correct it to restore the original working order.

Record the name of the person who verified the status along with the verification

date, time, item, and remedies taken to restore the original condition. This log should be presented to the Facility Utilization Director.

14) In case of an accident or an event that could lead to an accident

If a glass capillary is broken and the virus sample has leaked or is suspected of having leaked, disinfect and sterilize the area suspected to be contaminated and the experimental apparatuses without delay using glutaraldehyde and 75% alcohol. Items that can be sterilized with high-pressure steam should be so treated. If skin came into contact with the leaked virus sample, disinfect the skin with 75% alcohol.

If an accident occurs, immediately report it to the biological experiment principal investigator. The principal investigator should then immediately report it to the Facility Utilization Director and the head of the Safety Office.

The biological experiment principal investigator should provide remedial actions as outlined above in cooperation with the head of the Safety Office under the supervision of the Biological Sample Safety Manager.

15) Safety measures for specific biological samples in case of a disaster, such as an earthquake or fire

In the case of a disaster, such as an earthquake and fire, virus samples should be placed in a safe location so that no contamination occurs. Personnel who have been or might have been contaminated with virus samples should be rescued without delay. Emergency measures should be provided as prescribed in the previous section, *14) In case of an accident or event that could lead to an accident.*

Fires and injuries to personnel should be reported as prescribed in *Appendix 3, Emergency reporting.*

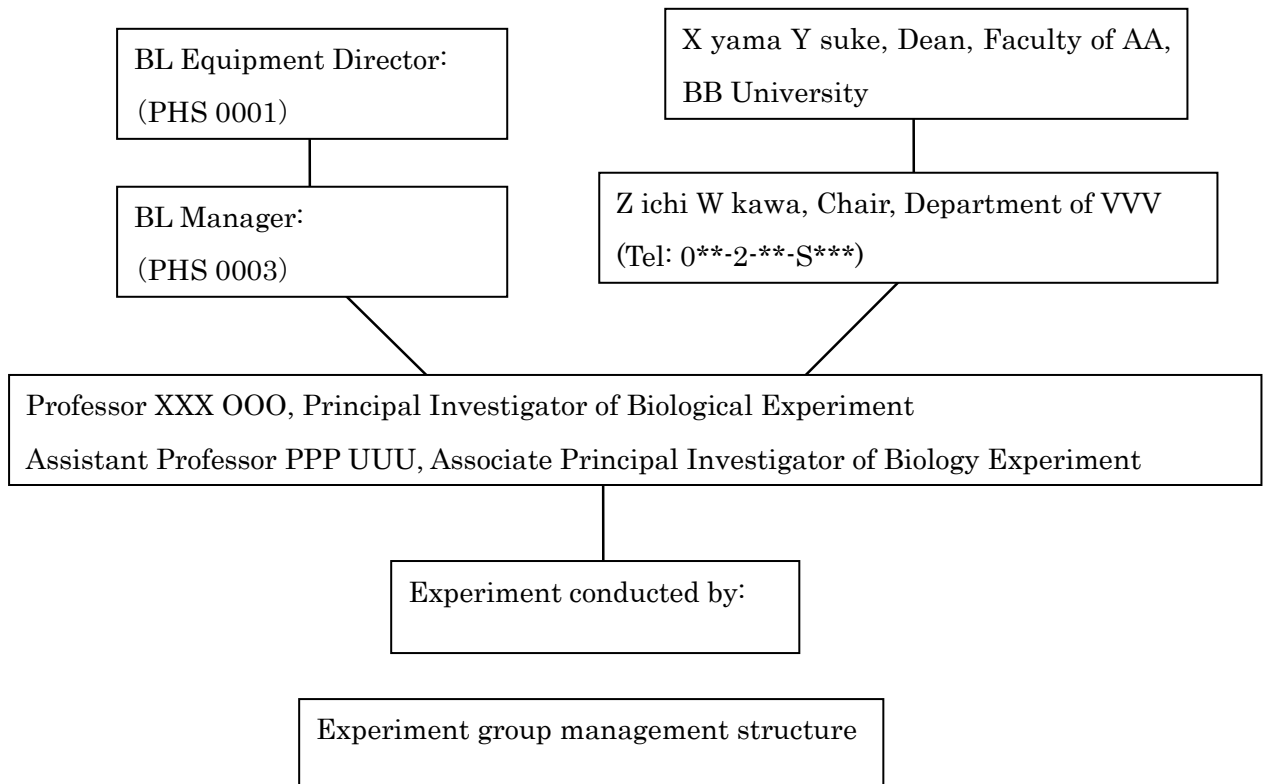
16) Entrance of outside individuals to BSL-2 Lab

Individuals who are not involved in an experiment but would like to enter the BSL-2 Lab should follow the instructions of the biological experiment principal investigator. The entry and exit of such individuals should be recorded.

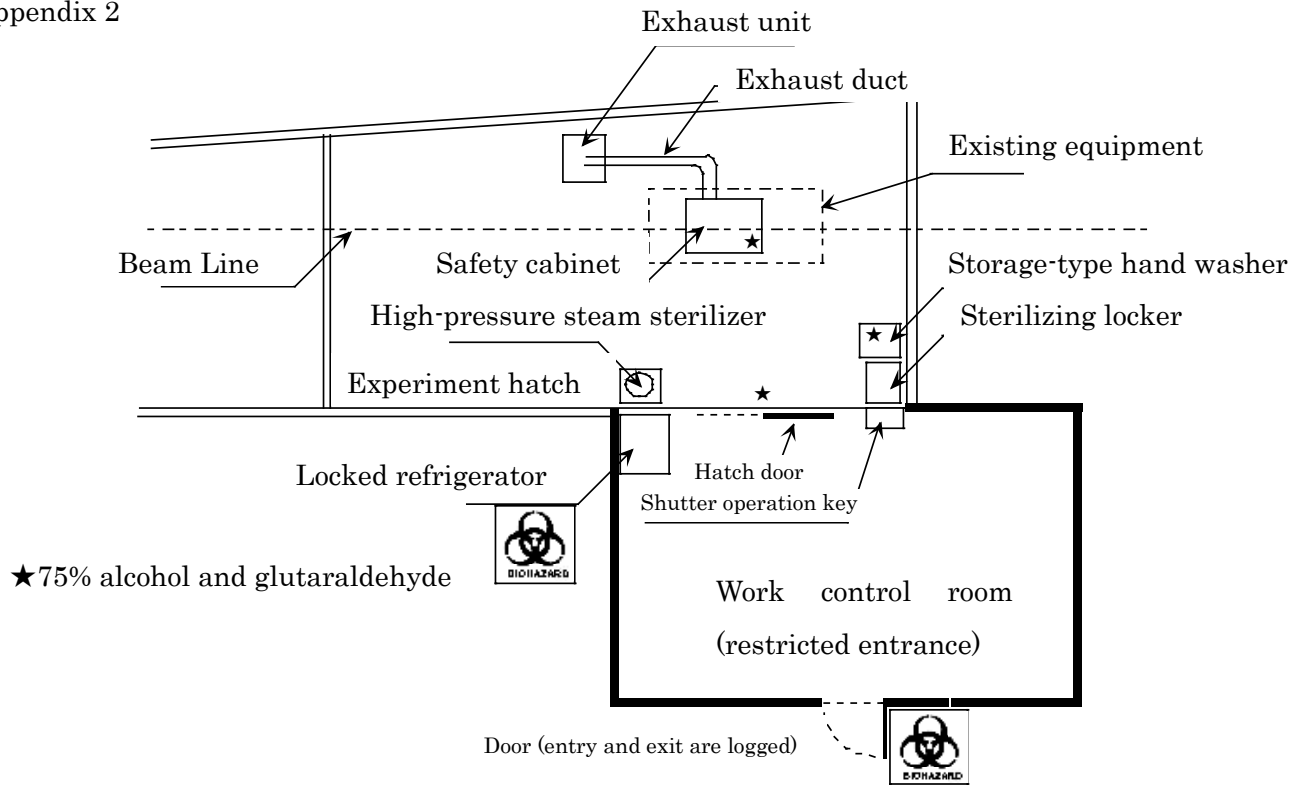
17) Other safety considerations

The emergency contact number of **** Laboratory, XXX Department, Faculty of YYY, ZZZ University, is 0* *-2* *-9* * * .

Appendix 1



Appendix 2



BL-1XU experiment hatch and work control room

Biological experiment inspection check list Biological experiment inspection check list (draft)

Director, Safety Office, SPring-8

I hereby submit my report in accordance with Clause 31-1 of Regulations on Biosafety.

Principal Investigator of Biology Experiment:

Prior to experiment: Date: Month: Year:

During experiment: Date: Month: Year:

After experiment: Date: Month: Year:

Inspections performed:	Issues discovered:	Remarks:

If an issue is discovered, please report it to the Safety Office (ext. 2200)