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Bernhard Nocht Institute for Tropical Medicine

STANDARD OPERATING PROCEDURE

Title	Operating a stationary glovebox
Short title	Stationary glovebox

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1 Purpose

The purpose of this standard operation procedure (SOP) is to describe how to operate a stationary negative glovebox, that allows the safe manipulation of samples potentially containing pathogens higher than biosafety level 2.

2 Scope

This SOP applies to all laboratory staff involved in processes that require working in the glovebox.

3 Abbreviations and definitions

Abbreviations and definitions included in alphabetical order

3.1 Abbreviations

BNITM	Bernhard Nocht Institute for Tropical Medicine
HEPA	High-Efficiency Particulate Air/Arrestance
µl	Microliter
ml	Milliliter
N/A	Not applicable
%	Percent
SOP	Standard Operating Procedure
VIR	Department of Virology

3.2 Definitions

SOP	A detailed, written instruction to archive uniformity of the performance of a specific function.

4 Responsibilities

Role/Title	Responsibilities/Tasks
Head of Laboratory	Release valid version of the SOP for use in the laboratory.
Designated senior laboratory staff	Take care of the regular review process for the SOP and supervise SOP and process specific trainings and the corresponding documentation.
All laboratory staff	Adherence to information given in this SOP. Daily cleaning of all workspaces, equipment and materials used.

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5 General description

The negative pressure glovebox is a containment workspace for the manipulation of samples of potentially containing pathogens higher than biosafety level 2. HEPA filters guarantee that the potentially contaminated air is filtered and thus ensures personal protection to the person manipulating the samples.

6 Device requirements

The glovebox needs to be set up on a flat, sturdy table, if no stand is included in the scope of delivery.

7 Material and equipment

7.1 General

- Negative pressure glovebox
- Pipettes: 20µl-, 200µl- and 1000µl- pipette and filter tips accordingly
- Working tray
- Tissue paper
- Spray bottle for 0.5 % chlorine solution
- Waste bag holder, waste bags and cable ties
- Racks for microcentrifuge tubes, 15 ml and 50 ml conical centrifuge tubes sample containers
- Pen resistant to 0.5 % chlorine solution

7.2 Maintenance

- Tissue paper
- Torch light
- HEPA filters

7.3 Storage

All equipment and material can be stored at room temperature.

8 Safety

Always wear a gown and gloves when working at the glovebox.
The operator will finally wear three pairs of gloves: nitrile gloves (primary), thick glovebox gloves (secondary) and nitrile gloves on top of the glovebox ones (tertiary).

9 Procedural description

9.1 Start-up process

9.1.1 Functional check

Before starting the work in the stationary glovebox, the following checks must be performed to ensure its functionality and personal safety:

- Glove check: check the gloves and sleeves for intactness. A torch can be used to thoroughly inspect the material – the light will shine through holes but not through intact material. Gloves or sleeves have to be replaced immediately in case of damage.

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- Pressure check:
  - Turn the glovebox on.
  - Check that the gloves are inflating due to the negative pressure.
  - The gloves can also be pulled out of the glovebox and will be pulled back to the starting position due to the negative pressure.
  - The glove might also have an integrated pressure gauge. Check that the pressure is within the expected range (check manufacturer's manual for specifications).

If a check fails, proceed to troubleshooting and do not start working in the glovebox before these functional checks pass after taking the requested measures.

9.1.2 Calibration

N/A

9.1.3 Validation

N/A

9.2 General principle

There are always two persons working at the glovebox: the **operator** and the **buddy**. The operator processes the samples in the glovebox, the buddy supplies the operator from outside of the glovebox with all reagents, consumables and samples through the airlock and he double checks that pipetting volumes are correct. He also documents all working steps on the laboratory request forms/ batch list.

The negative pressure is a necessary requirement to work at the glovebox and must be checked before starting to work, as well as the intactness of the gloves (see 9.1.1 – functional check).

0.5% chlorine solution is used as a disinfectant and has to be prepared. Please refer to corresponding SOP for additional information.

9.3 Good laboratory practice in the glovebox

- Only one sample should be processed/ opened at a time to avoid cross contamination
- All pipetting steps and opening of sample tubes are performed over the working tray
- The working tray is covered with a tissue soaked in 0.5 % chlorine solution.
- Used pipette tips must be flushed with 0.5 % chlorine solution (in a 50 ml centrifuge tube) at least 3 times before discarding into the glovebox trash
- Use a pen resistant to 0.5 % chlorine solution in the glovebox and to label all tubes, that will be processed in the glovebox

9.4 Pass box

Samples, reagents and consumables are introduced and exited through the pass box.
Whenever the pass box is opened from the inside, it must be disinfected by the operator by spraying the inside the airlock and the closing door/ lid with 0.5 % chlorine. Before opening the pass box from outside, an incubation time of 10 minutes must be watched.
At any time, the pass box may be either opened by the operator from the inside or by the buddy from the outside, but never at the same time. The operator and buddy must ensure this by proper communication.

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10 Waste management

All infectious waste, including leftover samples, tips and other used consumables are decontaminated with 0.5% chlorine solution before being discarded into the glovebox trash. The glovebox waste, together with all other laboratory waste, is discarded according to the national guidelines.

11 Maintenance

11.1 Internal

11.1.1 Daily maintenance

- Before starting the work in the glovebox, the functional checks (see 9.1.1) must be performed
- Fresh 0.5% chlorine solution must be prepared every day. Old 0.5 % chlorine solution must be discarded
- At the end of a working day, all trash must be exited from the glovebox. All surfaces, pipettes and other equipment should be wiped with 0.5 % chlorine solution

Record the completion of the daily maintenance on the respective maintenance form.

11.1.2 Yearly maintenance

All HEPA filters must be changed once a year. For instructions refer to the device manual. Make sure to decontaminate the old filters by dunking them in 0.5 % chlorine solution for at least 30 minutes and discarding them into the laboratory waste.

Record the filter change on the respective maintenance form.

11.1.3 Maintenance on demand

11.1.3.1 Glove change

Glove change is required, when gloves are not intact anymore.

- Check the new gloves for integrity
- Check that the glovebox is turned on
- Wear the new glove and slip inside the old glove
- Transfer the end of the old glove to the first notch of the ring, so that the glove is only hold by one of the two O-rings
  Take care: Do not lift the glove too high, so that air could move from inside out!
- Pull the new glove through under the first O-ring (that still holds the old glove)
- Pull one O-ring over the second notch of the ring to fix the new glove
- Carefully remove the old glove by pulling the glove out of the ring where it is fixed. Make sure to only pull the old, but not the new glove under it
- Discard the old glove in the infectious waste
- The new glove is now ready to use

11.2 External

N/A

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12 Troubleshooting

12.1 Pressure check failure

When the pressure check fails (see 9.1.1 functional check), do not proceed to work. Leave the glovebox switched on. Check that the pass box is closed properly and the gloves are well attached and intact.

12.2 Glove check failure

When a hole in a glove is detected during the functional check (see 9.1.1), the glove must be checked immediately. See 11.1.3.1 for instructions how to change gloves.

13 References

N/A

14 Associated Documents

14.1 Tools associated with this SOP

Glovebox maintenance form
Manufacturers manual

14.2 Other documents associated with this SOP

Process SOPs for:
Sample separation
Sample inactivation and RNA extraction

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15 Document History

Review date	Version number	Brief description of changes
N/A	V01	First release

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