

Expert information

from the Working Group on Hygiene

Information about handling techniques of individually ventilated cages (IVCs)

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Introduction

Individually ventilated cages (IVCs) are used for different purposes: hygienic separation of animals or animal groups, reduction of dust and allergen pollution in a room, reduced space requirements in so-called high-density caging systems or air-conditioning aspects. The emphasis of the following discussion is on the aspect of hygienic separation of animals. For this purpose, IVCs offer good protection against the transmission of infectious agents between cages. Single IVCs can act like completely separate hygienic entities with a self-contained barrier. This level of protection against contaminations, however, can only be guaranteed if the cages are handled correctly and are opened exclusively under the protection of a laminar air flow (cage changing station, laminar air flow cabinet). The following discussion is based on user experience, which should serve as useful information about the appropriate handling of IVCs. The techniques may also be applied to caging systems with static filter tops without active air exchange. In special cases, appropriate local conditions should be considered and changes should be done accordingly.

Cage changing station

The selection of a cage changing station depends on the activities of the animal facility in question and its safety evaluation (safety levels range from S1-S4 according to the risk potential of the biological organism being used [1-6]) and the genetic modification inherent to the animals. There are different types of laminar air flow cabinets available which can be used as an appropriate changing station [4, 5]:

- a) cabinets open to one, two or all sides
- b) class 2 microbiological safety cabinets (appropriate for use up to S3 safety level)
- c) closed class 3 microbiological safety cabinet isolators (appropriate for use up to S4 safety level).

In these cabinets, the laminar air flow can be directed inwards (user protection) or outwards (product protection). Cabinets for pure product protection are not suitable as cage changing stations because dust, allergenic or toxic contents and infectious agents should not be released into the environment due to hygienic and safety reasons in accordance with safety level 1. An appropriate cage changing station has a laminar air flow that is vertically oriented from the top to the bottom. This air flow prevents the introduction of infectious agents from the outside and it restricts contact of the user with dust or other substances from the working area. This kind of laminar air flow is suitable for both product and user protection.

With cabinets from category a) the air flows inside (front-side air barrier for user protection). Therefore, dust, allergenic or toxic substances and infectious agents are not released into the environment. Additionally, the laminar air flow in the working area of the cage changing station guarantees protection of the hygienic status of the animals. A suitable cage changing station has to also protect against the release of any aerosols, i.e., that both the laminar air flow inside of the cage changing station and the exhaust air have to be filtered through a HEPA filter (high efficiency particulate air filter class H14) [3]. Class 2 microbiological safety cabinets comply with the requirements of EN 12469 and guarantee that no infectious agents from opened cages are released into the environment and they are not transmitted from the outside into the cages due to the stronger air flow of the front barrier. Additionally, class 2 microbiological safety cabinets retain allergenic dust from opened cages in their filters and prevent their release into the environment. For work at safety level 3 and higher, additional precautions are required [4]. The present recommendations are limited for work up to safety level 2.

While selecting a cage changing station special attention should be paid to the properties of the front window system, ensuring that the opening is large enough for cages to be placed onto the working area. If hygienic requirements and safety level require the use of a class 2 microbiological safety cabinet according to DIN EN 12469 [3], the cabinet should be equipped with an adjustable front opening (e.g. by increasing or decreasing the height of the front window or a similar system) [5]. For the reduction of microbial agents, UV lamps may be used during breaks. There are cage changing stations available which comply with the specifications of class 2 microbiological safety cabinets and, as such, can be used in safety level 1 and 2 situations according to the genetic technology safety regulations (Verordnung über die Sicherheitsstufen und Sicherheitsmaßnahmen bei gentechnischen Arbeiten in gentechnischen Anlagen (GenTSV) [2].

Disinfectant

When working in a cage changing station the use of a liquid disinfectant is indispensable. Both the working area and the gloves worn should be continuously disinfected during the handling of cages to prevent possible transmission of infectious agents from the outside of the cages to the inside (or vice versa). This should also maintain the hygienic status of each single cage. The agent has to act quickly (within seconds) and has to be sufficiently potent. An appropriate disinfectant which fulfils all requirements (quick action, protection of staff, protection of material, range of effectiveness) is unfortunately not yet available. As suitable substances for prophylactic disinfection there are different acceptable agents which have been tested and are recommended by the Robert Koch Institute (www.rki.de), the Network of Applied Hygiene e.V. (www.vah-online.de) or the German Veterinarian Society (www.desinfektion-dvg.de). Often disinfectants are based on substances such as glutaraldehyde, chlorine dioxide (attention: chloric agents can damage stainless steel) or ethanol (attention: danger of explosion). Gloves being used should be pretested for disinfectability and impermeability after repeated disinfection with the disinfectant used [9]. Disinfectants can damage protective gloves due to degradation (= change in properties of material after contact with chemicals), penetration (= permeation of a chemical through microscopic pores) and permeation (= permeation at a molecular level) in a way that they will be useless (sticky, stiff or fragile) and their protectiveness will be nullified or alleviated [7-9]. When choosing

a proper disinfecting agent, all aspects including any potential side-effects on the animal (local and systemic effect) and any occupational health and safety regulations should be taken into consideration.

Preparation of IVCs

IVCs should be sterilized before use in order to maintain the hygienic status. Therefore, the cleaned cages are supplied with bedding, nesting material and, where appropriate, with enrichment, which are often autoclaved with a wire lid, divider and a fixed top all in one. However, it is not always possible to autoclave fully equipped cages due to lack of available autoclave capacity. Alternatively, the single items can be autoclaved separately in stacks, which are then moved under protective covers into the cage changing station. In the changing station, they are re-assembled (the top cage in every stack will be covered or not used because of possible contaminations). Sterile food and water bottles which have been autoclaved with content (in case there is no central water processing) are added under the changing station.

Changing IVCs

While transferring animals in a cage changing station the following steps should be followed:

1. All the equipment and material required in the changing station should be deposited sterile or disinfected prior to the commencement of work in the changing station. Containers or bags with sterile food and if appropriate with autoclaved enrichment have to be disinfected on the outside as well.
2. If bedding sentinels are used, a clean bedding sentinel cage must be placed in the changing station with suitable tools to collect bedding samples [10, 11].
3. All manipulations which require the opening of cages have to be done under the changing station. A clean autoclaved cage and a dirty cage with the animals inside should be placed side by side in the changing station. The dirty cage should be placed between the clean autoclaved cage and the sentinel cage ("hygienic gradient"). First, the clean autoclaved cage should be opened, followed by the dirty cage. The tops are put upright behind the cages against the backside of the inner working area or, if space is available, with the open side down beside the cages. Next, the wire lids can be removed and the animals are transferred into the clean autoclaved cage. If mice are transferred with cushioned forceps, these have to be disinfected after every cage change. To guarantee sufficient disinfection of the forceps, several forceps with different markings can be alternatingly used, which are then stored between usages in a container with a disinfecting agent. Next, enrichment (if) is put into the cage, the wire lid is replaced, food is put into the troughs, the top is fixed and the autoclaved water bottle added. After closing the new cage, the required bedding sample can be transferred from the dirty cage into the sentinel cage.
4. The gloves should be disinfected between each cage change or changed if required (e.g. necessary between handling of cages from different users/projects/racks or after soiling).
5. Cage changing can also be done by two people [12] reduce contamination risks.

Used IVCs

The bottom of used dirty cages should be transferred in closed containments to the respective washing area, including all other used parts of the IVCs. If complete cages are changed, dirty cages should be removed as closed units and should only be opened and emptied in the washing area. Cages from

infectious experiments or experiments of safety level 2 or higher must be autoclaved as closed cages before being opened and emptied outside of the changing station.

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