Upgrading the health status in a SPF facility without any renovation and downtime

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Introduction

The Institut Clinique de la Souris (iCS) is a research infrastructure for translational research and functional genomics. The iCS combines the capacity of generating Genetically Engineering Modified mice (GEM) on a large scale with a high-throughput and comprehensive phenotypic analysis of the animals. A 2100 m2 breeding facility is running to support iCS activities and provide mouse services such as microinjection, animal care, breeding, archiving and distribution.

16000 individually ventilated cages (IVCs) are currently used to house and to contain mice in 12 holding rooms. Our Health Status is unique through all the facility with endemic infection of some microbiological agents (*Helicobacter, Pasteurella, Norovirus* and other opportunistic agents).

However animals with higher microbiological quality are necessary for reproducible mouse models characterization. This scientific need may require for mouse facilities to be upgraded to improve the health status. But most of the time, upgrading facilities involves hard renovation, downtime and then important operational costs.

As a mouse production and banking centre for the scientific community, our challenge here is to improve mouse health and to generate mice free of *Helicobacter, Pasteurella and Norovirus* in a fully occupied and endemically infected breeding facility.

We previously tested a decontamination program by embryo transfer (ET) using sterile material in a usual housing room but it was not successful (transmission of microbiological agents 12 weeks after ET).

Then, we initiated a program to improve the health status of our mice strains based on rederivation through ET and use of Biobubble enclosure, a custom designed soft walled, and positive pressure class100 /Iso 5 clean room.

The Clean Room Project

1)iCS breeding facility design

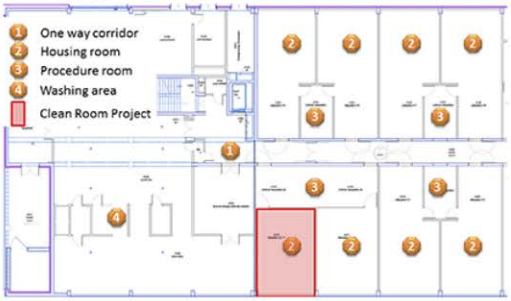

Fig1. Layout of the breeding facility (ground floor); Red square shows one the housing room where the Biobubble clean project room has been installed.

2) Biobubble Clean Room Principles and Installation

Biobubble clean room (*bioBUBBLE Inc*) is a soft walled positive pressure enclosure powered by 80-100 air changes per hour of HEPA filtration provided by Air Handling Units. Biobubble is simply constructed by hanging a vinyl skin with all Velcro-type connections over a tubular aluminium framework. This Biobubble enclosure has been installed in one of our housing rooms of the ground floor facility.

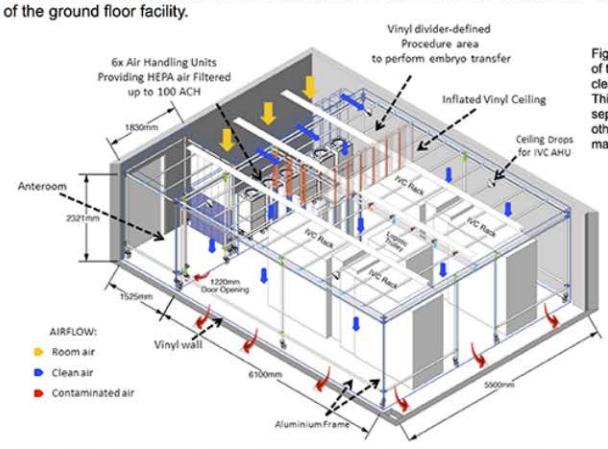

Fig 2. 3D conceptual of the Biobubble clean room project. This room is separated from the others in a air-proof manner

Workflow

To eliminate the undesired microbiological agents, we started in May 2013 to restock the Biobubble room by conducting a rederivation program through embryo transfer using the standard procedures. We rederived a variety of contaminated GEMs strains reflecting our current scientific effort at iCS. These strains are housed in endemically infected and adjacent rooms (see Fig. 1).

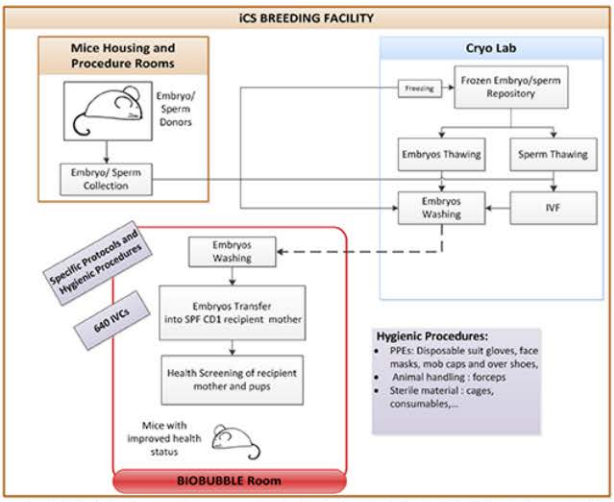

Fig 3. Flowchart illustrating the rederivation program into the iCS barrier with staff, material trafficking (between animal housing, Cryopreservation laboratory , and Biobubble rooms) and dedicated Hygienic procedures.

Embryos are recovered from contaminated donors strains in the procedure room. Embryos are then transferred in the Cryo Lab to be carefully checked for integrity and subjected to treatment and sequential washing procedures to be either frozen or transported into the Biobubble in order to be again sequentially washed in the procedure area (Fig. 2). Only zona-intact embryos are transferred to pseudo-pregnant SPF Crl:CD1 females purchased from Charles River Laboratories France.

Newly wild-type rederived mice (10 wks old), their recipient mother and CD1sentinels mice, are directly tested for *Norovirus, Helicobacter* and *Pasteurella* agents endemically present in the iCS breeding facility.

Results

Test date	Type of mice	Norovirus (ELISA)	Helicobacter sp. (PCR)	Pasteurella sp. (Culture)
October 2013	Foster mother and pups	0/30	0/30	0/30
March 2014	Sentinel mice	0/1	0/1	0/1
April 2014	Foster mother and pups	0/32	0/32	0/32
June 2014	Sentinel mice	0/1	0/1	0/1
September	Sentinel mice	0/1	Not tested	0/1
October 2014	Foster mother and pups	0/20	0/20	0/20
Total in Clean Room		**0/85**	**0/84**	**0/85**
Prevalence in iCS Breeding facility (01/2013-10/2014)		45/48 (94%) (Sentinels)	29/38 (76%) (Sentinels)	7/33 (21%) (colony)

Table 1: results of health analysis performed in house (IGBMC health monitoring service).

Microbiological agents to be screen are transmitted through feco-oral and aerosols. We therefore tested 3 animals per ET cage (recipient mother + 2 pups). We used as well 3 CD1 sentinel mice. This sampling of testing is sufficiently large to detect at least one positive mouse with regard to the prevalence of the 3 unwanted agents in our facility (Table 1). After the ET and a minimum of 10 weeks of housing in the Biobubble room, none of the 3 unwanted agents have been detected in the 35 recipient mothers and their 47 descendants screened. Each Sentinel mice exposed to dirty bedding during 16 weeks were also free of *Norovirus*, *Helicobacter* and *Pasteurella*.

Taking into account the high prevalence of these 3 agents, these results are very satisfactory and validate the rederivation program we set up with the clean room and robust husbandry procedures.

Conclusion

A lot of institutions have developed their own strategy to improve microbiological status of mice for animal welfare and scientific purposes. These strategies are highly depending upon infection issues, rederivation capabilities and most importantly barrier facility design and architectural constraints.

We presented here a straightforward program to eliminate *Helicobacter, Pasteurella and Norovirus* agents from our breeding facility through embryo transfer rederivation into a Biobubble, a soft-walled class 100/ISO 5 clean room. Biobubble room provides to our IVC-running facility a "containment within containment" in a very cost effective way allowing us to conduct rederivations in an adjacent but separate and contained room. Most importantly to set up this health upgrade, we avoided any temporary shutdown and hard renovation, and were able to continuously use the facility for our scientific programs.

This program to produce and to maintain mice with a higher microbiological quality in an endemically infected breeding facility is now fully used to meet specific scientific needs.