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MICROBIOLOGICAL VALIDATION OF THE M.I.C.E.[®] CAGING SYSTEM

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MICROBIOLOGICAL VALIDATION OF THE "M.I.C.E.®" SYSTEM

GOAL OF THE STUDY :

In addition to the original validations and studies already performed by G. Rivard or in Charles River - Iffa Credo (practical use and ergonomics, validation of the environmental parameters), we decided to re-assess the efficiency of the M.I.C.E.® system, to validate its efficiency when used for bio-exclusion in a contaminated environment or to house mice with heterogenous health statuses without cross-contamination.

DESIGN OF THE STUDY :

- The study was conducted between 14th September (T0) and 7th November 2000 (T105)

- Use of a double-sided M.I.C.E.® rack, with a 70 cage capacity (on each side : 5 cages per level, 7 levels). See appendix 1 for technical details.

- 3 OF1 and 2 nude mice (all outbred, female, 7 to 8 weeks old), with the SOPF health status (Specific and Opportunistic Pathogen Free : see appendix 2 for definition and origin) are placed in each "SOPF mice" cage. The nude mice are immunodeficient and more sensitive to bacterial and parasitic infections but do not seroconvert after an infection. The OF1 mice are fully immunocompetent and are used for viral antibody detection.

- 5 OF1 female mice, with the SOPF health are placed in each "challenge" cage.

- Mice nomenclature :
Ico : OF1 (Caw)
Ico : SWISS (N)-nu/nu

- Just after caging, the intermediate level cages, are challenged :

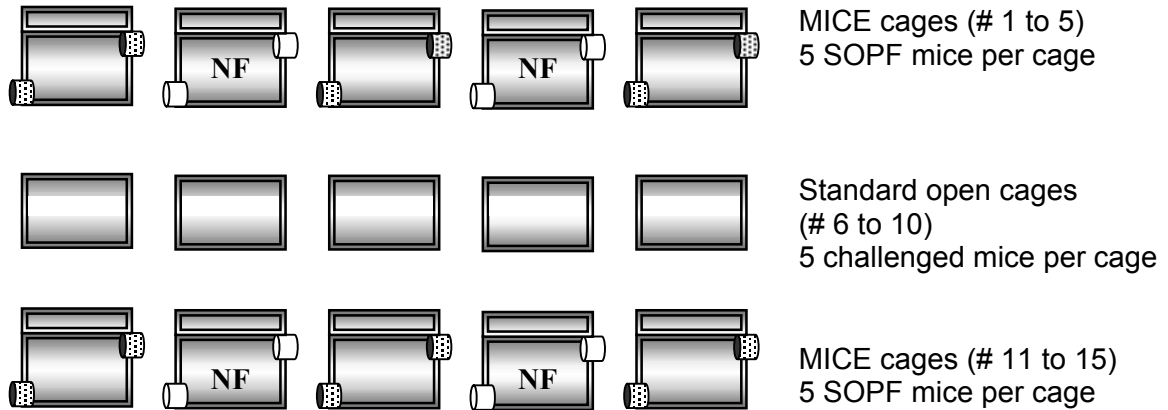
1. orally, in the drinking water, with a cocktail of opportunistic bacteria (*Pseudomonas aeruginosa* 5 10^{E7} CFU/ml, *Staphylococcus aureus* 5.6 10^{E6} CFU/ml, *Klebsiella pneumoniae* 10^{E7} CFU/ml, *Proteus mirabilis* 2 10^{E6} CFU/ml. See appendix 3 for details.

2. by the IP and IN routes with a MHV strain (Mouse Hepatitis Virus). See appendix 3 for details.

- The opportunistic bacteria were selected because of our long experience of opportunistic monitoring and particularly to their use as "marker" micro-organisms to assess a bio-exclusion system, their easy spread within an animal room when introduced, the possibility to detect them easily in the faeces (without stressing the animals or risking a contamination during handling), and in consequence, the possibility to conduct the whole study on a small number of animals, unchanged from the beginning to the end of the assay.

- A MHV strain was selected because, of the very high contagiousness, the quick spreading and the easy detection of this murine virus when introduced in an animal unit, despite its sensitivity to environmental conditions.

- In the central part of the rack, on 3 levels, 15 cages were positioned as described in the drawing :



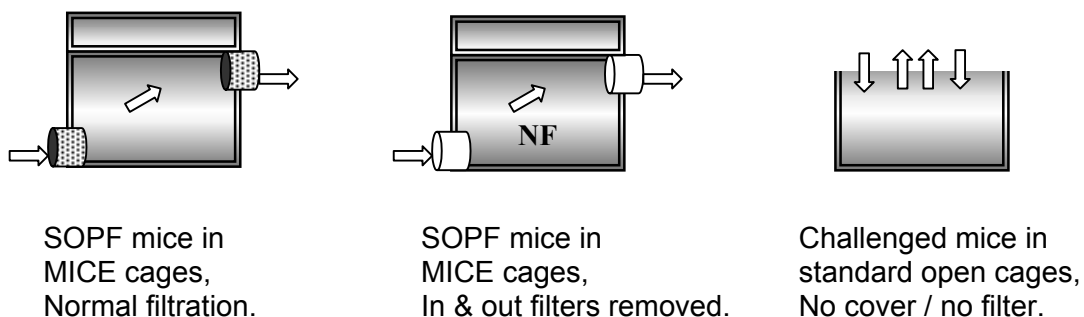
"NF" (for "No Filter") : MICE cages without in and out Reemay 2024 filter).

- The SOPF cages were position at the upper and lower level to be exposed both to descending (gravity, larger particles) and ascending (air convection, smaller particles) to airborne particles.

- The MICE rack air outlet (10 cm diameter flexible tube) was positioned close to the air exhaust of the room (50 x 50 cm), located at the level of the 3 lower rack levels.

- Some cages, marked NF (for "no filter", # 2, 4, 12, 14), the filtration media were removed. Only the grid piece was kept in place. This was done to assess a hypothesis formulated by the manufacturer which was an equivalent efficiency of the normal filtration device (Reemay 2024 filter in sandwich between 2 stainless steel grids) on the one hand, and of the 2 grids only on the other hand.

- Summary of the 3 caging situations (arrows indicates the air flow) :



- The racks and the laminar flow station were placed in a non-barrier air-conditioned room, isolated on site, but with a gowning procedure including a room dedicated overcoat, disposable surgery mask and cap and thick latex gloves.

- All cage parts and water-bottles were cleaned and sterilized before initiating the study (autoclave : equipment cycle, 121 °C / 20 minutes).

- All cages were supplied with sterilized feed (R03 formula, manufactured by U.A.R. France, gamma radiated at 2.5 Mrad) and with sterilized bedding (autoclaved 121 °C / 20 minutes), manufactured by S.P.S, France. Water bottles were filled with autoclaved water (in AMSCO, bottles for water autoclaving, water bottles autoclave cycle, plateau at 121 °C / 20 minutes).
- All handling, animal care, challenge administrations and sample collections were carried out in a laminar flow working station (manufactured by Faster, Italy, model specifically designed for rodent experimental handling: see appendix 1). This laminar flow working cabinet is specifically designed to benefit from a double protection : bio-exclusion (maintenance of the SOPF health status) bio-containment (protection of operator and environment against biohazardous manipulations).
- All handling and cage changing procedures were limited to once every 2 week-period in order to limit the risk of contamination during these operations and to give priority to the validation of the caging system rather than the handling and cage changing conditions.
- The standard Iffa Credo procedures for handling SOPF mice in filter-top cages were used, including spraying / scrubbing the gloves with ethanol (90 %) between each cage (i.e. each "dirty" and "clean step") and spraying the working surface and cages before opening each of them.
- Timing of the study (starting on 14 September 2000) :
 - Day 0 :
 - transfer of the SOPF mice into the cages
 - challenge administration (bacteria and MHV) in cages # 6 to 10
 - Day 5, 28, 49, 94, 105, in each cage :
 - sampling and screening for opportunistic bacteria : bacteriology on faeces sample (see technique in appendix 4)
 - sampling and screening for MHV : ELISA on serum samples (see technique in appendix 4), except on day 5 (need to wait for seroconversion time).
 - Day 105 : additional screening upon study termination : full serology (19 viruses and Mycoplasma), bacteriology, parasitology.

RESULTS :

Cage #	Cage type	Staph. aureus	Pseud. aeruginosa	T = 5 days		MHV
				Klebs. pneumoniae	Proteus mirabilis	
1	SOPF - STD	-	-	-	-	NT
2	SOPF - NF	-	-	-	-	NT
3	SOPF - STD	-	-	-	-	NT
4	SOPF - NF	-	-	-	-	NT
5	SOPF - STD	-	-	-	-	NT
6	CHALL	-	-	-	+	NT
7	CHALL	-	-	-	+	NT
8	CHALL	-	-	-	+	NT
9	CHALL	-	+	-	+	NT
10	CHALL	-	-	-	+	NT
11	SOPF - STD	-	-	-	-	NT
12	SOPF - NF	-	-	-	-	NT
13	SOPF - STD	-	-	-	-	NT
14	SOPF - NF	-	-	-	-	NT
15	SOPF - STD	-	-	-	-	NT

Cage #	Cage type	Staph. aureus	Pseud. aeruginosa	T = 28 days		MHV
				Klebs. peumoniae	Proteus mirabilis	
1	SOPF - STD	-	-	-	-	-
2	SOPF - NF	-	-	-	-	-
3	SOPF - STD	-	-	-	-	-
4	SOPF - NF	-	-	-	-	-
5	SOPF - STD	-	-	-	-	-
6	CHALL	-	-	-	+	+
7	CHALL	-	-	-	-	+
8	CHALL	-	+	+	+	+
9	CHALL	-	-	-	+	+
10	CHALL	-	+	-	+	-
11	SOPF - STD	-	-	-	-	-
12	SOPF - NF	-	-	-	-	-
13	SOPF - STD	-	-	-	-	-
14	SOPF - NF	-	-	-	-	-
15	SOPF - STD	-	-	-	-	-

SOPF – STD : fully equipped MICE cages, SOPF mice
 SOPF – NF : MICE cages without Reemay in & out filter, SOPF mice
 CHALL : standard open cages, SOPF mice challenged with opportunistic bacteria and MHV
 + or - : cage positive or negative for the screened microorganisms

Cage #	Cage type	T = 49 days				MHV
		Staph. aureus	Pseud. aeruginosa	Klebs. pneumoniae	Proteus mirabilis	
1	SOPF - STD	-	-	-	-	-
2	SOPF - NF	-	-	-	-	-
3	SOPF - STD	-	-	-	-	-
4	SOPF - NF	-	-	-	-	-
5	SOPF - STD	-	-	-	-	-
6	CHALL	-	-	+	-	+
7	CHALL	-	+	+	-	+
8	CHALL	-	-	+	-	+
9	CHALL	+	-	+	+	+
10	CHALL	-	+	+	+	+
11	SOPF – STD	-	-	-	-	-
12	SOPF – NF	-	-	-	-	-
13	SOPF – STD	-	-	-	-	-
14	SOPF – NF	-	-	-	-	-
15	SOPF - STD	-	-	-	-	-

Cage #	Cage type	T = 84 days				MHV
		Staph. aureus	Pseud. aeruginosa	Klebs. pneumoniae	Proteus mirabilis	
1	SOPF - STD	-	-	-	-	-
2	SOPF - NF	-	-	-	-	-
3	SOPF - STD	-	-	-	-	-
4	SOPF - NF	-	-	-	-	-
5	SOPF - STD	-	-	-	-	-
6	CHALL	-	-	-	+	+
7	CHALL	-	+	-	+	-
8	CHALL	-	+	+	+	-
9	CHALL	-	-	-	-	+
10	CHALL	-	-	-	-	+
11	SOPF – STD	-	-	-	-	-
12	SOPF – NF	-	-	-	-	-
13	SOPF – STD	-	-	-	-	-
14	SOPF – NF	-	-	-	-	-
15	SOPF - STD	-	-	-	-	-

SOPF – STD : fully equipped MICE cages, SOPF mice
SOPF – NF : MICE cages without Reemay in & out filter, SOPF mice
CHALL : standard open cages, SOPF mice challenged with opportunistic bacteria and MHV
+ or - : cage positive or negative for the screened microorganisms

Cage #	Cage type	T = 105 days				MHV
		Staph. aureus	Pseud. aeruginosa	Klebs. pneumoniae	Proteus mirabilis	
1	SOPF - STD	-	-	-	-	-
2	SOPF - NF	-	-	-	-	-
3	SOPF - STD	-	-	-	-	-
4	SOPF - NF	-	-	-	-	-
5	SOPF - STD	-	-	-	-	-
6	CHALL	-	-	+	+	+
7	CHALL	-	+	-	+	+
8	CHALL	-	+	+	+	+
9	CHALL	-	-	+	+	+
10	CHALL	-	-	-	+	+
11	SOPF - STD	-	-	-	-	-
12	SOPF - NF	-	-	-	-	-
13	SOPF - STD	-	-	-	-	-
14	SOPF - NF	-	-	-	-	+
15	SOPF - STD	-	-	-	-	-

SUMMARY : CUMULATED POSITIVE RESULTS OVER THE DIFFERENT TESTS

Cage #	Cage type	GLOBAL	T = 105 days			MHV
		Staph. aureus	Pseud. aeruginosa	Klebs. pneumoniae	Proteus mirabilis	
1	SOPF - STD	-	-	-	-	-
2	SOPF - NF	-	-	-	-	-
3	SOPF - STD	-	-	-	-	-
4	SOPF - NF	-	-	-	-	-
5	SOPF - STD	-	-	-	-	-
6	CHALL	-	-	++	++++	++++
7	CHALL	-	+++	+	+++	+++
8	CHALL	-	+++	++++	++++	+++
9	CHALL	+	+	++	++++	++++
10	CHALL	-	++	+	++++	+++
11	SOPF - STD	-	-	-	-	-
12	SOPF - NF	-	-	-	-	-
13	SOPF - STD	-	-	-	-	-
14	SOPF - NF	-	-	-	-	+
15	SOPF - STD	-	-	-	-	-

Additional testing on day 105 : complete screening (serology with 20 antigens, bacteriology, parasitology) of SOPF cages. Result : no animal tested positive.

SOPF - STD : fully equipped MICE cages, SOPF mice
SOPF - NF : MICE cages without Reemay in & out filter, SOPF mice
CHALL : standard open cages, SOPF mice challenged with opportunistic bacteria and MHV
+ or - : cage positive or negative for the screened microorganisms
+ to ++++ : each "+" represents the number of tests for which the cage has been found positive over the total study duration.

CONCLUSIONS :

The bacterial and MHV challenge inoculations appeared to be successful for all agents in all "challenge" cages, except for *Staphylococcus aureus* (only one positive result on day 49).

It appears clearly that for the testing period, with the techniques used and under the study conditions :

- we couldn't evidence any sign of contamination of the animals other than the agents used for the challenge inoculations,
- all fully equipped MICE cages didn't show any contamination during the 105 days (15 weeks) following the challenge inoculations in the open cages kept on the same rack,
- this is equally true for the "NF" cages during 84 days. On day 105 but one of these cages was found positive for MHV on day 105. This is invalidating the hypothesis that the stainless steel grids are as efficient as the full air filtering device (Reemay 2024 filter in sandwich between 2 stainless steel grids).

In conclusion, if adequate handling procedures are used, it appears that the M.I.C.E. system is efficient to manage the housing of different health statuses in the same room for a period of time of at least 3 months / 15 weeks.

In theory, an other study should be initiated to assess the long-term efficiency of the system. However, for longer durations, one may expect to assess the handling conditions as being the limiting factor, rather than the efficiency of the caging system.

12th June 2001

M.I.C.E. CAGE

External dimensions: $38 L \times 19.8 W \times 24 H$

Internal dimensions: $34.3 L \times 17.8 W \times 16.5 \text{ to } 21.6 H$

Floor area: 610.5 cm^2

Material:

Macrolon^R (Bayer) "Hi-Heat" polycarbonate

Cage components:

Cage and cover,

Drop-in feeder, water-bottle and cardholder,

Enrichment design.

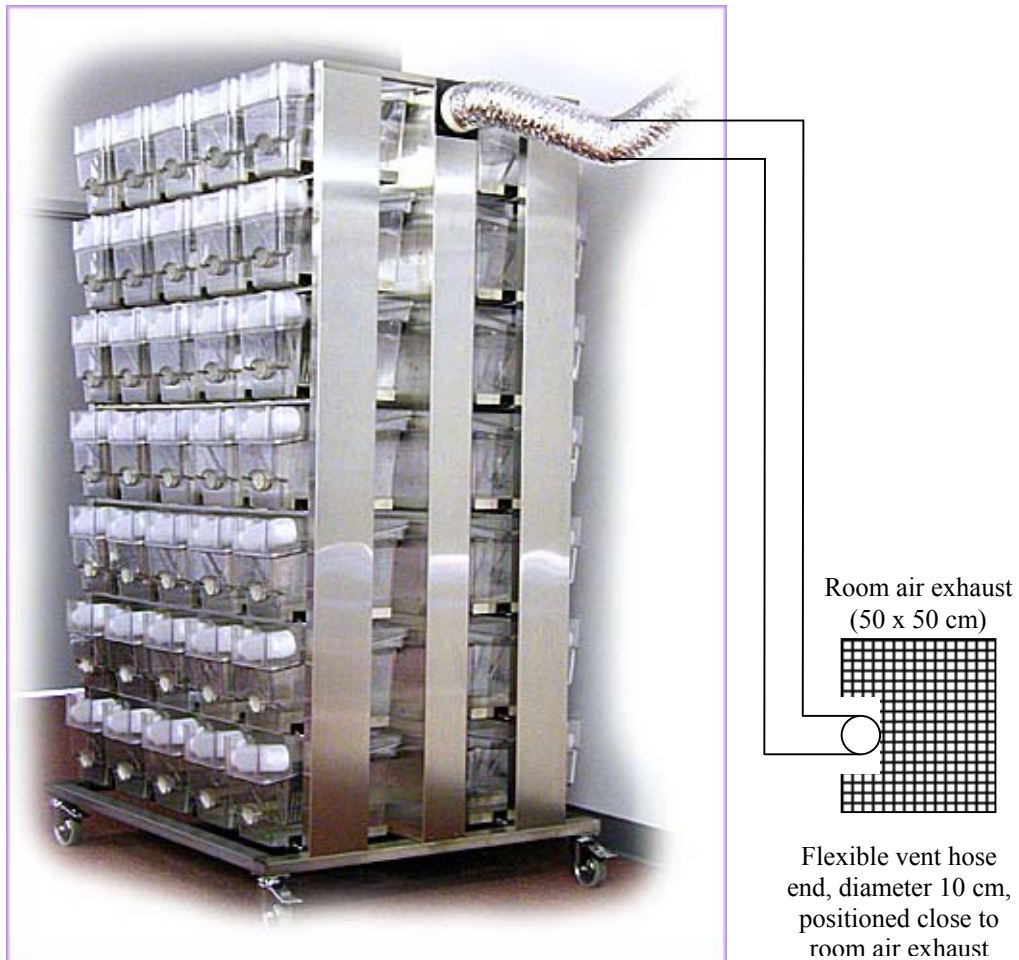


M.I.C.E. CAGE

- **Closed system:**
 - **bio-exclusion and health status maintenance**
 - **protection of users: allergens**
- **Non mechanical ventilation system: convection**
- **Ventilation:**
 - **10 to 60 RPH, by convection**
 - **low air velocity**
 - **air filtration at cage level, in and out, stainless steel grid and Reemay 2024 filter:**
 - **validation data available upon request (temperature, humidity, NH₃, CO₂,...)**



Rack used for the study (70 cages / double sided):

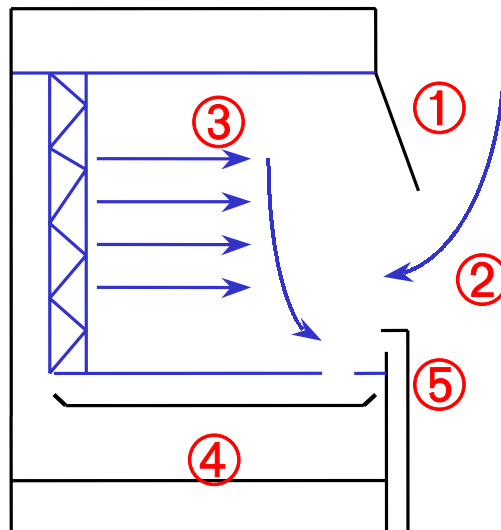


Air distribution:

Contemporary Topics, Jan. 2000, 39; 1, pp. 22-27



1. Improved visibility for handling
2. Enlarged front opening for easy animal and cage handling
3. Horizontal flow for optimal bio-exclusion when opening a filter-top or MICE cage, but still compatible with biohazard working conditions
4. Easy bedding and particle collection and cleaning
5. Working surface front edge designed to avoid dispersion of particles out of the hood



PROTECTION OF MANIPULATION AND ENVIRONMENT
Special Hood Design for Animal Modèle spécial ANIMALERIE

APPENDIX 2

DEFINITION OF THE SOPF HEALTH STATUS

In Charles River - Iffa Credo, immunocompromized and genetically modified mouse lines can be bred and maintained under SOPF / SSC^{UP} health status (*Specific and Opportunistic Pathogen Free*, in adaptation and translation of the original wording in French : *Statut Sanitaire Contrôlé pour Utilisations Particulières*).

This health status was initially developed by Iffa Credo in 1983 under isolators to breed mouse strains with genetic (nude then scid, bg) or induced immunodeficiency (γ radiation) or to guarantee the absence of the major interfering opportunistic agents, in addition to the specific pathogens.

In 1987, for capacity, cost and flexibility reasons, a filter-top cages / laminar-flow-working station system was validated and is used successfully since that year.

Because of the specific issues related to the development, breeding and maintenance of genetically modified rodent lines, this technique was selected by our Transgenic Services Department.

Several hundred thousand mice have been bred successfully with this system (mutants, inbred and genetically modified lines).

Each filter-top-cage-unit is monitored on a weekly basis for opportunistic agents by bacteriology.

Within the same filter-top cages unit, a full bacteriology / parasitology screening plus partial serology is carried out every month and a complete health screening every quarter.

Additional details on the health monitoring procedures, techniques and sampling methods are available upon request.

Globally, the SOPF / SSC^{UP} definition for mouse breeding projects includes the screening of the following agents :

<u>Viral infections</u>	<u>Bacterial infections (1)</u>	<u>Parasitological infections</u>
MVM (<i>serology</i>)	Tyzzler's disease (lesions)	Ectoparasites
MHV (<i>serology</i>)	Bordetella bronchiseptica	GI tract endoparasites
PVM (<i>serology</i>)	Citrobacter freundii 4280	
MPV (<i>serology</i>)	Corynebacterium murium	
Sendaï (<i>serology</i>)	Mycoplasma pulmonis (<i>serology</i>)	
TEMV/GDVII (<i>serology</i>)	Pasteurella sp	
Reo 3 (<i>serology</i>)	Salmonella sp	
Ectromelia (<i>serology</i>)	Streptobacillus moniliformis	
Hantaan (<i>serology</i>)	Streptococci β haemolytic (2)	
LCM (<i>serology</i>)	Streptococcus pneumoniae	
	Helicobacter hepaticus (PCR)	
	Staphylococcus aureus (3)	
	Pseudomonas aeruginosa (3)	
	Proteus spp (3)	
	Klebsiella pneumoniae and oxytoca (3)	

(1) Bacteriology except if an other technique is mentioned.

(2) Except Streptococci β haemolytic of D group which are classified as opportunistic agents.

(3) Main opportunistic agents according to FELASA recommendations, negative results guarantee not only the absence of the 4 mentioned agents but are also a relevant indicator for the absence of human-born opportunistic agents.

Additional agents can be screened upon request.

The monitoring also includes the reporting of any clinical sign and/or any lesion observed during the necropsies and, in case of finding, the histopathological examination results.

APPENDIX 3

CHALLENGE INOCULATIONS

Bacterial strains and challenge :

- Pseudomonas aeruginosa : ref CRF 1000078, mouse isolate
- Staphylococcus aureus : ref ICO 1001531, mouse isolate
- Klebsiella pneumoniae : ref D/141, rabbit isolate
- Proteus mirabilis : ref ICO 1002219, mouse isolate

4 Trypticase Soybean medium tubes are inoculated with the bacterial strains (1 tube per bacterial species) and incubated for 12 hours at 37°C, 24 hours before challenging the mice.

The content of each tube is transferred aseptically into 1 liter of sterile distilled water.

Counting is carried out on each water preparation to determine the viability of the bacteria and the number of CFU (Colony Forming Units) per ml at T0.

Results :

- Pseudomonas aeruginosa : $5.0 \cdot 10^7$ CFU/ml
- Staphylococcus aureus : $5.6 \cdot 10^6$ CFU/ml
- Klebsiella pneumoniae : $1.0 \cdot 10^7$ CFU/ml
- Proteus mirabilis : $2.0 \cdot 10^6$ CFU/ml

The water obtained is used as drinking water on T0 to the mice of the challenge cages (# 6 to 10), after removing the drinking water overnight between T -1 and T0.

MHV virus and challenge :

MHV (Mouse Hepatitis Virus, Mouse Coronavirus) strain ref MHV-A59 WSV 3-19-93, frozen suspension of $4.0 \cdot 10^7$ TCID₅₀ received in June 2000 from Charles River Laboratories Diagnostics, kept at - 80°C until day of challenge.

At T0, the viral suspension is thawed and diluted 1/40th in sterile isotonic saline solution, immediately prior to inoculation.

Then, the MHV suspension is immediately used to challenge each mouse of cages # 6 to 10 : they are inoculated with 0.5 ml by IP route and 1 micro-droplet by IN route.

APPENDIX 4

SAMPLING AND SCREENING TECHNIQUES

SAMPLING :

On days 5, 28, 49, 94, 105 for the 4 opportunistic bacteria : *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Proteus mirabilis*.

On days 28, 49, 94, 105 for MHV.

On day 105 for the full SOPF screening : parasites, bacteria (pathogenic + opportunistic) and virus serology (see annex 2 for the full list of agents screened).

TECHNIQUES :

Bacteriology :

According to the technique developed and used for more than 15 years for "routine opportunistic bacteria screening" , collection of fresh faecal pellets from each SOPF MICE cage (3 OF1 and 2 Swiss-nu/nu per cage), samples pooled per cage.

Culture on selective media :

- Pyocyanic bacillus Selective Medium for *Pseudomonas aeruginosa*,
 - Baird-Parker for *Staphylococcus aureus*,
 - Eosin methylene blue for enterobacteria : *Proteus mirabilis* and *Klebsiella pneumoniae*,
- All selective media manufactured by BioMerieux, France.

Serology :

Blood collection from the immunocompetent mice (OF1) of each SOPF cage (individual sampling) and serum preparation.

Serum used for MHV ELISA test, Charles River Laboratories standard kit : plate and reagents (conjugate and controls), MHV strain ref. MHV-A59 WSV 3-19-93.

Full screening on day 105.

Iffa Credo techniques and procedures identical to standard health monitoring, quarterly screening (parasitology, bacteriology and serology). See screening list and technique used in annex 2).

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