UNIVERSITÀ DEGLI STUDI DI PALERMO	**Standard Operating Procedure (SOP)**	Document Code: POS-02 Revision Number: 0 Pag. 1 of 7 Annexes: 4

Title: MICROBIOLOGICAL CONTROL OF SURFACES AND ANIMALS OF THE ANIMAL HOUSE OF THE DEPARTMENT OF BIOPATHOLOGY AND MEDICAL AND FORENSIC BIOTECHNOLOGY

Changes to previous versions:		
Date	Ref. Point	Reason for the change
Changes are highlighted underlined in the procedure		

Rev.	Issuing Date	Preparation	Technical Verification (RBA)	RQ Verification	Approval Director of the Dept
0	15/04/2014	Guido Sireci			
1					
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MICROBIOLOGICAL ENVIRONMENTAL CONTROL (ANIMAL HOUSE)

1. PURPOSE

This document describes the procedures for monitoring animal health and environment housing in order to ensure the safety of operators and animals.

The housing of the animals and the management of the premises and equipment contained therein is carried out in compliance with the POS (Operating Procedures Standard) currently in use.

2. REFERENCE DOCUMENTS

- These procedures were written in accordance with Legislative Decree n. 26 of 4 March 2014, Annex III.

- European Commission Recommendation of 18 June 2007 on guidelines for the accommodation and protection of animals used for experimental and other scientific purposes.

- Filippo Pasquinelli (1981) - Diagnostics and Laboratory Techniques - Rossini Editrice- vol II.

- R. Ligugnana: Environmental microbiological control. Application Notes, 1999 International

PBI, Milan.

- FELASA recommendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit colonies in breeding and experimental units. Lab Anim. 2014 Mar 4.

3. PROCEDURES OF ENVIRONMENTAL SAMPLING

3.1 MICROBIOLOGICAL CONTROL OF AIR

This document provides information related to sanitary control of the air in order to guarantee healthy conditions for the animals, as well as the operators and to remove the risk of transmission of zoonoses.

The air can be a vehicle of contamination, for this reason the animal house is equipped with air treatment systems with various levels of efficiency that minimize the presence of microorganisms.

It is therefore necessary to constantly monitor these areas to evaluate the effectiveness of the treatment systems. It is necessary to validate the air treatment system and ensure its proper functioning with the Monitoring.

The type of contaminants is represented by molds, bacteria, viruses, dust and pollen. The most common bacteria present in confined environments are: Bacillus, Pseudomonas, Staphylococcus, Micrococcus, Methylobacterium and Flavobacterium. These microorganisms are part of the normal microbial flora of the confined environments and their presence in general must not create alarmism.

Validation:

It consists of carrying out quarterly air sampling in preset critical areas, to verify the limits set by the SOPs. During the environmental monitoring the following microbiological parameters are determined:

1. Total bacterial load at 36°C (mesophilic bacteria): optimal development temperature between 25 and 40°C. In this category are placed the conventional pathogenic bacteria and all the bacteria that make up the normal flora of man. It is a generic index of air contamination.

2. Total bacterial load at 20°C (psychrophilic bacteria): optimal development temperature between 15°C and 30°C. This category includes all the saprophytic microorganisms that are able to perform their life cycle at the expense of decomposing organic substances. Therefore, they are able to colonize the soil and the humid environments.

3. Total mycetic load: the mushrooms of interest include mold and yeast. The determination of the mycetic parameter in environmental samples is necessary because it often relates to the presence of dust and at the same time can be remarkable in presence of high humidity. It is also considered a generic index of air contamination.

4. Staphylococcus spp.: they are Gram positive cocci and are numerically the most represented in the normal microbial population of the human skin and oropharynx. It is an indicator of good cleaning practices and pathogenic potential.

5. Coliforms: they are a group of rod-shaped bacteria, Gram-negative, asporigenous, aerobes and facultative anaerobes, which ferment lactose, with gas and acid production, at 35-37°C (in 48 hours) and possess the β-galactosidase enzyme. They are ubiquitous organisms generally present in faecal material, and therefore can be used as indicators of pollution. The group includes species belonging to different genera, including the sub-group of fecal coliforms.

6. Streptococci: genus of gram-positive bacteria of spherical shape (cocci). They are facultative anaerobes and catalyse negative (a useful feature for the differential diagnosis of Staphylococci). They are able to produce toxins; one of which is capable of destroying red blood cells. This characteristic allows them to be classified according to the type of hemolysis they produce when they are grown on blood agar plates. Many species are part of the bacterial flora present in humans.

7. Haemophilus: this genus includes Gram-negative bacteria with marked pleomorphism (bacillary, coco-bacillary or filamentous forms), immobile, asporigenous, aerobic-anaerobic facultative. Fresh blood is needed for their development on culture media.

The method used for the microbiological control of ambient air is based on active sampling using the "Surface Air System" (SAS) solid surface impact sampler. An alternative method used for the microbiological control of the ambient air is based on passive sampling which involves the use of sedimentation Petri dishes.

Active Sampling

The SAS sampler aspirates a known volume of air, conveying it into a Petri dish containing agar; the particles dragged by the current, by inertia, impact on the surface of the agar by depositing itself. As many aliquots must be taken as the types of microorganisms to be found; each aliquot contains a selective agar, different according to the type of microorganism to be isolated.

The count of bacterial colonies of the plates is carried out after incubation at temperature and time appropriate to the microorganism being tested, expressed in u.f.c./m^3.

Passive Sampling

According to the Microbiological Contamination Index (IMA), the Petri dishes are arranged for the time of 1 hour, at 1 meter of height and at 1 meter from each obstacle. Such Petri dishes contain different solid culture media based on the type of microorganism to be isolated. The plates used will be ICR plates (contact plate irradiated with γ-rays).

The procedure involves the following steps: The lid of the Petri dish containing the soil is removed, so that the surface of the agar remains exposed to the air for a defined time (1 hour). At the end the plate is closed and the incubation proceeds. After microbiological monitoring of the air, the colony counts that may be present in the plates (IMA index) will be carried out according to this procedure: count the number of grown colonies, each of which represents a particle carrying microorganisms fallen on the surface of the agar. Results are expressed in unit of measurement: UFC (= Colony Forming Unit/m^3). The number of UFCs found is the IMA index.

Culture media

- SDA + CAF (Sabouraud Dextrose Agar + Chloramphenicol) medium for isolation and identification of yeasts and molds.

- BPA (Baird Parker Agar) medium for isolation and identification of Staphylococcus spp.

- VRBA medium (Violet Red Bile Agar) for coliform isolation and identification.

- Agar - Blood for isolation of Haemophilus.

- Slanetz Bartley Agar - for isolation of streptococci.

LIMITS OF ACCEPTABILITY OF THE TOTAL MICROBIAL LOAD OF THE AIR

ENVIRONMENT	**S.A.S. (U.f.c./m^3)**	**I.M.A. (U.f.c./m^3)**
Laminar flow hoods	< 1	< 1
Surgery room for immunocompromised animals	< 125	< 39
Surgery room for immunocompetent animals; premise with cages of immunocompromised animals	< 250	< 84
Other environments	< 375	< 124
NOT ACCEPTABLE	< 375	< 124

Non-compliant results are shown in the appropriate table (annex 1).

3.2 MICROBIOLOGICAL MONITORING OF SURFACES

The purpose of this operating procedure is to describe the modalities of the sanitary control of the surfaces in order to guarantee health conditions for the animals and for the operators.

MICROBIOLOGICAL ENVIRONMENTAL CONTROL (ANIMAL HOUSE)

The microbiological controls allow to trace the profile of the microbial flora present in the environment; they allow to identify the so-called 'critical points' and are necessary for the identification of possible sources of contamination and the possibility of cyclical phenomena. This strategy includes a periodic health check, as reported in Annex III of Legislative Decree 4 March 2014, № 26.

The surfaces to be taken into consideration during sampling are:

1. Cages, grids, test animal toys, baby bottles

2. Hoods, surgical instruments

3. Workbenches, shelf holders

4. Trolleys, shelves, stools, cupboards

5. Aluminum doors, handles, frames, push-button panels

6. Floor and walls

The systems used for the microbiological monitoring of surfaces require the use of pads and contact plates. Contact plates can be used for smooth surfaces. These are plates with a diameter of 55 mm (surface 24 cm^2) and a grid bottom. They are filled with agar to form a convex surface.

Sampling is performed as follows:

- Remove the outer casings and select an appropriate number of plates.

- Remove the cover from the plate.

- Place the side with the agar on the surface to be sampled, pressing lightly for about 10 seconds.

- Close the plate with the lid; storage is at 4°C until analysis.

The media used for contact plates are:

• Plate Count Agar (PCA): Total bacterial load.

• MacConkey\VRB Agar: Coliforms.

• SDA + Chloramphenicol: Molds and yeasts

The plates will be incubated at 37°C for 48h and at 25°C for 5 days. The non-conforming results are reported in the appropriate table (annex 2).

Specific tampons are used for irregular surfaces or points that are not easy to reach.

The pads consist of a rigid stem (in plastic) and a soft head (in cotton, synthetic fiber or alginate). They are packaged in test tubes and are included in a neutralizing solution, in order to neutralize the effect of disinfectants used for cleaning surfaces.

Sampling is performed as follows:

- Delimitation of the area to be sampled with a sterile mask (typically 10 x 10 cm^2).

- Extraction of the sterile swab from its casing.

- Humidification with the appropriate solution; elimination of any excess liquid by pressing the swab on the tube wall.

- Sampling of the area under examination by swiping the swab horizontally, vertically and diagonally for about 20 seconds; the pad should be rotated so that the whole head comes into contact with the surface.

- Insertion of the buffer into the test tube containing the chosen medium (neutralizing solution or transport medium).

- Immediate smear on plate or storage at 4°C until analysis.

The checks are carried out quarterly.

The acceptability limits for the surfaces described above are between 1 and 50 CFU/cm²).

The result is expressed as:

№ of colonies: Judgment;

From 0 to 25: Excellent;

From 26 to 50: Good.

Exceeding 50 CFU/cm² the result is not acceptable.

Monitoring, control and recording of environmental conditions and work surfaces must be performed according to the environmental control plan (annex 3)

4. VERIFICATION OF THE STERILIZATION EFFICACY OF THE AUTOCLAVE

The verification on the successful sterilization must take into account the fact that the it occurs through several factors: pressure, temperature, time and humidity, and are therefore diversified according to the parameter to be evaluated. The check of the equipment (autoclave) is undoubtedly the starting point: a malfunctioning autoclave, as well as an ineffective drying due to too short times, can compromise the final outcome.

In addition to the verification of the equipment, for steam autoclave sterilization, there will be checks provided through process indicators, as well as biological sterilization indicators.

The purpose of these controls is to verify the effective ability to kill microorganisms.

Each sterilization cycle involves the use indicator tape of sterility; quarterly the effectiveness check is performed using the bacillus stearothermophilus spores at the concentration of 10^5.

5. MICROBIOLOGICAL MONITORING OF ANIMALS

The purpose of this operative instruction is to describe the procedures for checking the parasite load in endo- and ecto-parasites, serological screening, in order to guarantee both the safety of the operators and the protection of animal health, as well as verifying that they are ensured adequate care for standard sanitary maintenance that conforms to the species raised in the facility.

MICROBIOLOGICAL ENVIRONMENTAL CONTROL (ANIMAL HOUSE)

Colonies of immunocompetent animals are monitored through serological screening performed by the health monitoring center of the supplying company Charles River, on fecal and blood samples collected and sent by us. The Charles River company provides the same service from **genetically modified and immunodeficient animals**, according to a sample collection process which provides the guidelines to be followed.

In particular, a serological screening will be requested to the company for the detection of a panel of micro-organisms to be agreed from time to time with the veterinarian. We will use PCR techniques combined with serology using EZ Spot-cards in order to increase the sensitivity for some agents present in low amount, and at the same time, contain and decrease the budget commitment compared to the use of sentinel animals. The followed protocol - PRIA - (PCR Rodent Infectious Agent) proposed by the Charles River company can be consulted in annex (Annex 4).

Animal monitoring will be performed annually.

Attached health check card (annex 4)

MICROBIOLOGICAL ENVIRONMENTAL CONTROL (ANIMAL HOUSE)

Annex 1

AIR MONITORING - NON-CONFORMING SAMPLES

DATE:

SAMPLING SITE:

PLATE READINGS:

UFC/m^3

SDA + CAF

BPA

VRBA

Blood agar

SBA

NOTE:

TECHNICIAN:

Annex 2

MICROBIOLOGICAL CHECK OF SURFACES – NON-CONFORMING SAMPLES

DATE:

SAMPLING SITE:

SURFACE:

PLATE READINGS:

UFC/m^3

PCA

VRBG

SDA

NOTE:

TECHNICIAN:

Annex 3

Annual Plan of the Microbiological Check – Year…….

Month	Microbiological check of air	Microbiological check of work surfaces
January		
February		
March		
April		
May		
June		
July		
August		
September		
October		
November		
December		

DATE

_____

Signature

_____

Annex4

charles river

Mouse Serology Profiles

Agent*	Parvovirus	Prevalent	Tracking	Assessment	Assessment Plus
MPV** (1-5)	●	●	●	●	●
MVM	●	●	●	●	●
Generic Parvovirus NS-1	●	●	●	●	●
MHV**		●	●	●	●
MNV		●	●	●	●
TMEV (GDVII)		●	●	●	●
EDIM (ROTA-A)		●	●	●	●
SEND			●	●	●
PVM			●	●	●
REO			●	●	●
MPUL			●	●	●
LCMV				●	●
MAV**				●	●
ECTRO				●	●
K				●	●
POLY				●	●
MCMV					●
HANT					●
PHV (HANT)					●
MTLV					●
ECUN					●
CARB					●
LDV					●
Sample Suitability Control: Tissue	●	●	●	●	●
Sample Suitability Control: Anti-Mouse IgG	●	●	●	●	●
System Suitability Control: Mouse IgG	●	●	●	●	●

**Agent abbreviations are defined in the Glossary of Terms.*
***Multiple assays are included. MPV: several recombinant viral coat proteins (VP2) to detect seroconversion to MPV-1 through -5. MHV: a recombinant MHV nucleocapsid (N) protein and two highly purified whole-viral lysate antigens. MAV: highly purified whole-viral lysate antigens to both FL and K87.*
****Price per serum sample when submitted directly to the serology laboratory.*