

# Environmental Microbiological Monitoring of Minipigs for Biomedical Research

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Production and Supply of Minipigs for Biomedical Research (<http://minipigs.angrin.tlri.gov.tw/>)



## Foreword

Microbial infection, in addition to the health impact of miniature pigs, microbial infection can also cause the formation of lesions in body organs and cause abnormal physiological function, thereby affects the validity of biomedical experiments. The sources of infection are either mainly from environment or from animal itself (endogenous). Therefore, to maintain good health quality miniature pigs for biomedical research, strict biosecurity implementation, strengthened self-defense, epidemic prevention, to prevent the invasion of pathogenic microorganisms off, but should also be the venue pigs health and environmental microbial monitoring, out with the original pigs and cut environmental pollution . Health monitoring including quarantine, and environmental pathogens check. Microbial monitoring consists of the effect of cleaning and disinfection of premises, sterilization function validation of autoclave, drinking water quality examination, and microorganisms monitoring of feed.

Microbial detection needs technology and specialized equipment. We perform autoclave function validation and the effect of cleaning and disinfection of premises ourselves, the detection of potential pathogen in drinking water and feed are contracted out to the certified professional laboratory.

## Pigs Health Monitoring

Indicators: as we produce the minimal disease pigs, regular monitoring of the following specific pathogen of pig diseases (see table):

- (1) Porcine reproductive and respiratory syndrome (PRRS).
- (2) Post weaning multi-systemic wasting syndrome (PMWS).
- (3) Classical swine fever (CSF).
- (4) Foot and mouth disease (FMD).
- (5) Pseudorabies (PR).

Once these five malignant diseases infected, the pigs will lead to significant harm and loss.

The veterinarians check pig premises three times daily for pig's health status and doing necessary treatment. According to the vaccination plan, pigs are regular received injections for classical swine fever, foot and mouth disease and pseudorabies vaccine (see technical documentation TAPS-TD-007). Four serum specimens, male and female each, are randomly taken and sent to specific laboratories for antibody examination from vaccination each season yearly. If suspicious death or abortion cases were found, the specimens will be then sent immediately for disease diagnosis. As we do not introduce any pigs from the outside, quarantine operations are not implemented, however, a "standard operating procedures for pig quarantine measures" is available when needed.

## Feed microbes monitoring



*Drinking water filtration and chlorination disinfection equipment*

Indicators: Salmonella are not allowed to be detected positive and Aflatoxin 50 ppb or less. Our feed is purchased regularly from contracted manufacturers. One kilogram each batch of feed is sent to a professional certified laboratory for quality check. The test items include the crude protein, crude fat, acid value and Aflatoxin content. The feed should look bright light yellow, not sticky when making crumb, with grain or dairy product flavor. If moldy, putrefied, foul-smelling odor or other microbial contamination is suspected, it should immediately prohibit feeding to animals and send sampling for inspection.

**Drinking water microbes monitoring**

Indicators: by filter membrane assay, drinking water containing coliform bacteria less than 6 cfu (colony forming unit) per 100 milliliters; and the total number of colonies (total bacterial count) less than 100 cfu per milliliter; Salmonella shall not be detected.

We provide mountain spring water for drinking and cleaning after filtration and chlorination disinfection. The chlorine residual and the cleaning of water supply pipeline are checked daily. Maintenance of chlorination equipment is done annually. Also, more than five random sampling points from the raw water, chlorinated water

reservoir and drinking nipples are sent to specialized laboratory for testing. The test items contain eight kinds of heavy metals and microorganisms content.

**Autoclave sterilized in validation**

Indicators: by use of chemical indicator tape, completely sterilized is confirmed through color changes (white stripes to black) and biological indicator(BI) each season yearly.

Many opportunistic microorganisms such as Streptococcus, Staphylococcus, Pseudomonas aeruginosa exist in the environment can cause infection through unclean injections or castration surgery wound. Therefore, the injection needle and surgical tools, etc. shall be autoclaved. The sterilization temperature is above 121 °C, with a

Autoclave sterilized in validation



pressure of 1.06 kilograms per square centimeter or more, the heating time is 15-30 minutes (heating time is adjusted depending on the amount of articles, and the item is not placed over two-thirds of the capacity). More than 30 minutes of heating is required for all the biological materials waste from laboratories. We do regular maintenance to ensure proper functioning of our autoclave, moreover, for every batch of sterilization, we use chemical indicator tape to ensure complete sterilized (right temperature and pressure) through color changes (white stripes to black). (as shown in the figure) .All biological waste materials arising from the experiment should be dumped after autoclaving.

#### **The effect of cleaning and disinfection of premises confirmation**

Indicators: after evacuating all the pigs, smear cultures by using eosin methylene blue (EMB) agar



*smear cultures*

are performed from five sampling sites on the premises bed, and the total coliform generated should less than 100 cfu.

After evacuating all the pigs on the premises, perform cleaning and disinfection and finally by flames. After 30 minutes, randomly select five sampling points of interior front, back, right, left in bed, by using a sterile cotton swab, smear a

diameter of 90 mm EMB agar plate per sample. Take fresh feces swab, smear as controls . EMB agar are put into the  $35 \pm 1$  °C incubator, and culture for  $48 \pm 2$  hours to observe whether specific colonies are generated. The specific coliform colonies are rounded, convex, smooth, neat edges, dark blue with a metallic sheen.