

### CAM assay Troubleshooting Tips and Tricks for fungal contamination

#### In ovo / Ex ovo

Fungal contamination is a problem, which has an impact on the outcome of the experiment. Sometime the fungi are not harmful for the embryo; however, we do not dare to use this result. The fungi contamination in CAM assay is visible in form of black powder or green fluffy traces inside the eggs. In this letter, we would like to share some tips from our experience to fight against fungal contamination.

**Appropriate disinfectant** and incubation time are important for preventing and eradicating the contamination. 70% ethanol seems to eliminate only bacteria while has less effect on fungi. Using disinfectant, which contains anti-fungal agents, such as Bacillo® AF could better affect fungi contamination. Importantly, the exposure time after spraying disinfectant must be long enough to kill fungi and their spores. Fungi will be killed after 5 and 15 minute, respectively. Do not dry out disinfectant with towel paper! Therefore, we recommended here to **clean the incubator, all equipment and working surface with disinfectant** containing anti-fungal agents. However, since we do not know the effect of this disinfectant on the chick embryo, we **do not use it for the eggs**. If the incubator is dirty from egg residues, we remove the residues with water before cleaning with disinfectant. From our experience, we recommended to **use plastic racks** instead of paper racks since plastic material is able to be washed with water and clean with disinfectant.

Dirty eggs could be the source of fungi contamination. If there are broken eggs during the delivery, dirty eggs need to be washed with water and dried out with towel paper before transferring them into the incubator. Using high volume of ethanol to clean the dirty eggs instead of water **is not recommended**. A small amount of ethanol for wiping the eggs a bit should be fine.

Furthermore, suitable **waste disposal** is implemented. Waste of contaminated eggs need to be well packed into the plastic bag, put into well closed container and removed from the experiment room as soon as possible to avoid the spread of the fungal spores.

Finally, **personal hygiene** after working in contaminated condition is necessary. The person who worked in the contamination area needs to clean himself or herself properly by showering and washing his/her hair before working again the next day.

We hope that you do not face with fungi contamination problem, but if you have it, our tips would be beneficial for you. Feel free to share your experience with all of us. Send emails, we will collect and transfer to our CAM community members.

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