

## **Sterilization and Disease Control**

### **By Nigel Venters and Glenn Baines**

#### ***Nigel Venters*** ***Plant, Facility and Livestock Care***

##### The Egg Wash

The Monarch egg collection and egg wash procedure by Melanie McCarthy and Terry Terbush is provided at the end of this article, with photos and instructions.

I use Terry and Melanie's method, and the only difference is that I use regular strength Milton Solution in place of bleach in the egg wash. However, Milton is strong against bacteria, O.e. spores, etc., but is not so corrosive to the eggshell as bleach. I give them 10 to 15 minutes in the Milton dip solution. The timing is not so critical. Of course, with bleach you have to time it right, or the eggshell melts!

##### Plants

I use Milton Solution directly on plants and they are unaffected by this. I use a hand-held mister for smaller cages or boxes, and a large spray bottle with a shoulder strap and a tube running to a plastic tube with a misting nozzle at the tip.

##### Equipment, Surface Areas

Everything is the same as for plants. Everything gets a good misting once a week. Of course, every day we do a swipe wipe down, for all work surfaces. Remember, we are constantly using anti-bacterial gel on our hands, between working with cages or boxes to avoid cross-contamination. We also use quite a lot of neat alcohol in a spray for a fast turn-around, as it evaporates so quickly, but of course is deadly to pathogens.

##### Interior of Flight House

Nature is a good provider here, but we do like to take action with a yearly deep clean and plant cut-back. I pull back the overhead shade, and this allows the sunshine to penetrate fully. Then in the evening, I mist everything with Milton Solution using the large sprayer. After the cut-back, it is a good time to check for ants, etc., that may have been missed when all the plants were rampant.

## Hygiene

My working “uniform” consists of a T-shirt and shorts for pretty much most of the year here. I have many sets of them (basically old clothes)! Standard in the pocket is a small bottle of gel sterilizer. We also do use surgical gloves, especially with some potent host-plants. When we have stock in the isolation area, I always just go straight inside my house, put my clothes in the washing machine and have a shower and change, before I move to any breeding area.

This probably sounds a bit over the top, but I just get on with it. When I catch new stock, I spray neat alcohol onto the net and gel my hands between each butterfly I catch. They go into brand new, sterile envelopes. All frass goes into a plastic “frass bin” which has changeable plastic bin liners. This is then put into the trash. Of course, all frass from sleeves also goes into the bin. Sleeves are put into the washing machine after a brief spray with Milton. We use a hot wash, but no detergent.

## Disposal of Stripped Down Milkweed Plants

Stripped Milkweed plants - I dispose of these plants by first spraying the soil with Milton, pulling the plants out and re-spraying. The plants are put into a bin liner for disposal. The soil is fertilized and re-planted with fresh seed immediately. I never compost stripped host-plants!

## ***Glenn Baines***

### ***Disinfecting Agents for the Laboratory***

#### Chlorine

Chlorine (chlorine hydroxide or common household bleach) normal concentration is 4%. This product has pungent fumes that are bad for the lungs, and can cause dizziness and nausea. Prolonged and frequent exposure can lead to "Occupational Asthma."

*Must be diluted to use it safely... common concentrations:*

10% ratio of bleach to water for serious cleaning purposes (1 part concentrated to 9 parts water).

5% ratio of bleach to water for sterilizing eggs, plants, cages (1 part concentrate to 19 parts water)

*That can be calculated as follows:*

5% ratio of bleach to water concentration = (.04 x 30 ml bleach) + 370 ml water. So for practical use, mix one gallon of 4% bleach with 13.3 gallons of water.

Chlorine, when diluted properly can be a safe disinfectant. It is effective at killing bacteria, breaks down fast, and is dispersed when exposed to air and sun.

#### Oxine AH

Oxine has been proven effective against a broad spectrum of *bacteria, mold, mildew* and *fungi*. When prepared according to label instructions, **Oxine** <sup>(AH)</sup> eliminates microorganisms of animal health concern in minutes or seconds, rather than the long contact times required with other sanitizing products. Mix 1 part oxine to 6 parts water, put solution in a humidifier to create a sanitizing vapor that will come in contact with everything in the lab. Put a fan behind the humidifier to better distribute the vapor.

#### Peroxide

The normal concentration of Peroxide is used for cleaning purposes. Peroxide is not as effective as chlorine, but doesn't have toxic fumes. Alternative Use: mix 1 part Simple Green with 1 part Hydrogen Peroxide.

#### Milton Solution

Milton sterilizing fluid is produced by [Procter & Gamble](#) for sterilization uses. It contains 1% [sodium hypochlorite](#) (NaClO) and 16.5% [sodium chloride](#) (NaCl). 1:80 dilution is used to sterilise babies feeding utensils and a 1:20 solution is [isotonic](#) with body fluids. 1:4 dilution is used for wound management applications. This contains 0.25% w/v available [chlorine](#) and has a [pH](#) of 10.5 - 11.2.<sup>[1][2]</sup>

Using Milton mixed 1:8, would be equivalent to a 12.5% solution of household bleach (3% concentration). We use 10% bleach solution for washing totes, cages, utensils, surface areas, etc.; so that is similar to Milton. (we use at 5% for eggs and host plant washes).

Milton actually contains 16.5% NaCl, or table salt. So, mixed 1:8, that would be a 2% salt solution. So Milton can be made at home using these mixture ratios.

### 96% proof Ethanol (Everclear)

Dilute to 40% and put into a spray bottle. Use to spray cages and work areas every day.

### Max Mixture

Mix this product using 1 part isopropyl alcohol, 1 part ammonia mixture, and 4 parts tap water. Ammonia and alcohol will not react, and it will kill anything (besides us). This has an unpleasant odor, and you should not use it in confined spaces.

Additional use for Ammonia: Restore and enhance the wing color of Painted Lady or similar butterflies by exposing it to some weak fumes of ammonia for a few minutes.

## ***Norms and Procedures for Lab Use***

### General Instructions

1. The entrance to the laboratory is restricted to staff authorized by the **Head of the Lab**.
2. Do not come into the lab with dirty shoes/boots.
3. Wash your hands with antibacterial soap before handling any specimen, **NO EXCEPTIONS**. Then put on your rubber gloves before working.
4. Only the **Head of the Lab** can authorized the handling of any specimen.
5. Eating, or handling food, is not allowed **inside** the Lab.
6. You are not allowed to bring in any Tools, biological material (fruit, plants, food, etc), that have not been authorized by the head of the Lab.
7. All the Lab Tools that are used, daily, must be washed and sterilized with Chlorine at 20%, after finishing the tasks (scissors, brushes, shelves where one works with the larvae and the ones to keep the boxes).

### Hygiene of the Cages, and Plastic Boxes for Reproduction

1. Once the cups have been used, they must be washed with blue soap, and the lids with chlorine at 20%.
2. Put them to drain until the following day.
3. Sterilized the cages according to the sep up protocol.  
The cages, if necessary, are washed with soap and water, then submerged in chlorine at 10% during 1 hr minimum, but do not leave until the following day as the metal ring goes rusty. Rinse and let it dry.

### Hygiene of the Floor and Lab Surfaces

1. The floor must be maintained in perfect hygiene. Floors need to be washed with chlorine before going out for lunch.
2. The *inorganic rubbish* must be thrown out into the especial bin assigned for this purpose.
3. The *organic rubbish* must be thrown out during the process, into a plastic bucket, and then take it to the **assigned area**.
4. Sweep and mop the floor **every day**. Mop with chlorine, taking special care to the corners.
5. When the temperature is 25°, or more, inside the lab, switch on the fan. **Do not leave the fan on all night long.**
6. When the temperature is less than 25°, close the shutters and door.

### Hygiene of Towels, Material or Pads

1. Mainly use Kitchen paper towels.
2. The pads are mainly used for drying one's hands. The towels that are used when one is changing larvae are only used for that purpose. The pads are then washed with chlorine at 20%.

**Monarch Eggs and Egg Sterilization**  
**By**  
**Melanie McCarthy and Terry Terbush**

This article presents techniques and recipe for washing and sterilizing Monarch eggs, to eradicate O.e. infection at the very beginning of the lifecycle and raising process. The recipe presented below for the egg sterilization solution was amended to be correct using a stronger concentration of bleach products, just introduced to the market place (2013).

Recipe:

Take as many Monarch eggs off your host plants as you need to sterilize.

**Note:** Unlike a lot of other species eggs, Monarch eggs are quite thick skinned, and you can gently roll them off the host plant quite easily without damaging them. It's best to leave the eggs on the leaves for 24 hours after being laid, before you remove them to sterilize them. This will allow the egg shells to harden before you remove them from the host plant.

Gather all your eggs, and gently drop them into a strong screened coffee filter. Next, immerse them in egg sterilizing wash solution, follow the instructions.

If you need to hold the eggs back from hatching, you can put them in the refrigerator in an airtight container until you are ready, but for no longer than 7 days. Eggs will take lower temperatures in a fridge than adults, but do try and make sure the temperatures are at least 5° C., (41° f.) or above.

Here is how to make the egg sterilization wash solution in undiluted form. It is a bleach and water solution, and different butterfly farmers have experimented and used different percentages of bleach. This is what has worked for us: if using bleach with active ingredient of 8.5% - 9%: use two cups bleach to nine cups of water. Add two or three drops of Ivory Liquid dishwashing detergent -- which makes the eggs very slippery so that they come right off of the leaves. After you make up this batch of concentrated bleaching solution, when washing the eggs, dilute by using 19 parts water to one part of solution (above) to make enough to wash that day's eggs. One breeder uses 1 tablespoon of the concentrated solution to 19 tablespoons of water. This gives you plenty of egg wash for one treatment.

### Method:

Put your final solution into a glass bowl that will accommodate the copper colored filter with the eggs already in the filter. Turn your timer on for 7 minutes. Immerse the copper colored filter into the diluted egg wash solution. Agitate the eggs for 7 minutes, *constantly*. Use a turkey baster to spurt solution onto the eggs, which will cause a lot of agitation of the solution all over the egg, on top of the egg, the sides and the bottom. After 7 minutes, remove the filter from the solution and rinse the eggs for 3 minutes, three different times, in clean bowls of water. Use the same filter, the same turkey baster. For the Finale, perform the Egg Swirl.

### Egg Swirl:

As you lift up and remove the coffee filter out of the water (with the washed and now rinsed eggs), swirl the mixture around so that about 1/2 the eggs swirl to 1/2 of the filter and are scattered up and down the side, then turn the filter over and dunk the other half back in so that the remaining eggs swirl over the other half of the filter. Voila!

Now lay the filter down on a clean/sterile surface covered with a napkin or paper towel which will instantly begin to absorb the excess liquid, until the eggs dry. It will take about 4-6 hours for the eggs to dry enough. Then pick up the filter and tap the sides with a utensil. All the eggs drop to the bottom. No sticking or mushing of eggs! And best of all, you only use 1 tablespoon of your concentrated solution per day. Remember, the egg wash solution is concentrated so before you actually use it on your eggs, further dilute it by 19:1. This is 19 parts water; 1 part concentrated solution.

After the eggs are dry, pour them into a sterile (tiny) container or souffle dish, breathe on them to add some moisture back since the bleach can be drying, and then cover with an airtight lid. Let them hatch out completely. Keep only the same age eggs together. Do not mix eggs laid from different days as you want all the eggs in this vial to hatch out at the same time. When they hatch out, you can either add a leaf or two of milkweed so they can crawl onto this or better yet take a 6-oz. sterile cup and stand up some milkweed in the cup. Turn over your container with the newly emerged larvae and tap on it with a heavy butter knife. All the new larvae will fall onto the milkweed in the next larger sized cup.



Glass bowl



Copper-colored coffee filter



Eggs sitting in solution ready to be agitated



Eggs being agitated with turkey baster



Bowl with solution and turkey baster alongside



Draining...



Eggs left in filter



3 bowls of clean water to rinse the eggs 3 times at 3 minutes each using turkey baster





After the eggs are swirled around the coffee filter, they are laid onto a paper towel or napkin to dry for about 4 - 6 hours



Tiny cup to store clean eggs