

## Egg inoculation with PBS and harvest of allantoic fluid

**Material Required:** 3 eggs, 18G needle, syringe, 1XDPBS, egg disposal box, falcon tube, scissor, tweezer

### Personal Protective Equipment

1. Lab Coat closing on the back
2. Sleeve protectors (one time use only)
3. Lab shoes
4. Shoe covers (one time use only)
5. Gloves + stich protection gloves
6. Freshly prepared 0.5 % incidine



Figure 1 Eggs, stich protection gloves, 18G needle, syringe, tweezer, scissor.



Figure 2 The eggs would arrive in cartonnage, it would be transported into the hood using the transport box shown above.



Figure 3 Transfer the eggs to the biosafety cabinet using transport box, while transferring clean the eggs and the transport box with meliseptol.



Figure 4 Tilt the egg facing the air sac, use torch to identify the spot and mark it with marker for the injection site.



Figure 5 Wear the stitch protection gloves in the left hand and take the 18G needle in the right hand. Make a small hole in the marked area of the injection site.

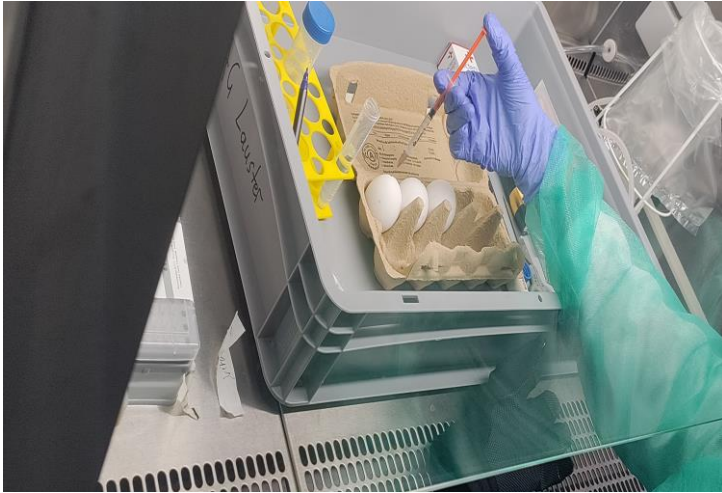


Figure 6 Fill the syringe with PBS and inject the PBS to the site where small hole was created using one hand.

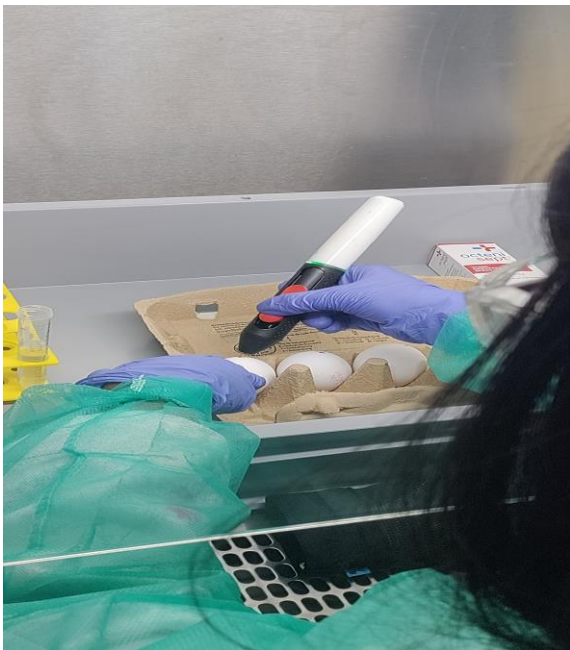


Figure 7 After injecting PBS to all the eggs, fill the holes by using glue gun.



Figure 9 Transfer the eggs back to the chicken egg incubator using transport box.

### Harvesting of allantoic fluid



Figure 1 Remove the wax from the eggs using tweezers.



Figure 2 For harvesting of the allantoic fluid, remove the egg shells using scissor. Use only one hand for harvesting process to avoid any injury.



Figure 3 Remove the outer covering of the egg gently by using tweezers.

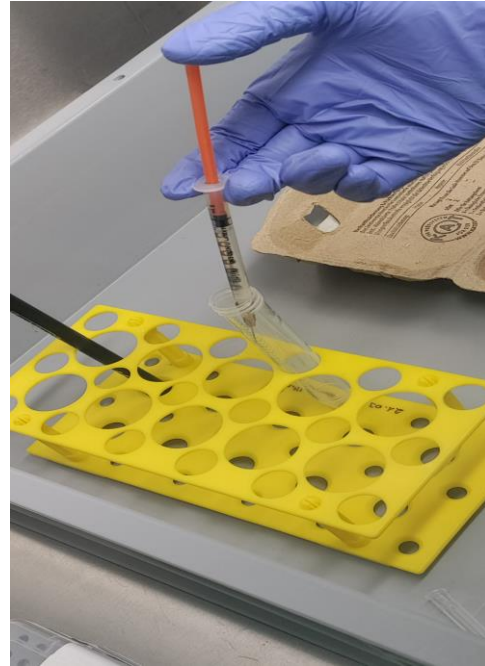


Figure 4 Harvest the allantoic fluid using syringe and transfer it to 15 ml falcon tube.

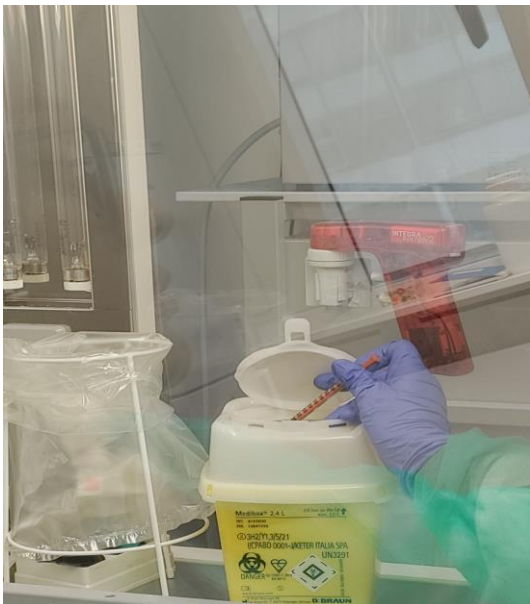


Figure 5 Discard the syringe and the needles in the appropriate waste bin. Keep the waste bin near the hood while performing the egg inoculation and harvesting process.

## Eggs disposal:



Figure 6 Auto clave the infected eggs and then the eggs will be transferred to a box labelled with egg disposal and stored at  $-80^{\circ}\text{C}$ . The cartonnage will be autoclaved and discarded after the egg harvesting process.