

## **Safe removal of animals from an isolator and transfer into microisolation (MI) cages using Trans Disks™ (TD).**

### **Introduction:**

Transfer disks (TDs) can be entered into an isolator for removing animals to microisolation (MI) caging by either introducing multiple TDs sterilized inside a standard “isolator supply cylinder” via a transfer sleeve, just like other isolator supplies, or, by autoclaving TDs individually and introducing them in smaller numbers just through the entry port of an isolator.

### **Method A.**

Introducing sterilized TDs “off the shelf” into an isolator for animal transfer to MI caging outside the isolator. After autoclaving individual TDs with their holes open and allowing them to cool, the ventilation holes should be taped shut and then the TDs can be stored indefinitely until needed.

If the “wetness” of your steam autoclave is high and the final drying cycle does not completely dry the filter medium, simply increase the drying time or allow the TDs to dry at room temperature as the filter medium is hydrophobic and any residual beads of water on top of the filter will dry at room temperature above, then the holes can be taped shut.

- 1) Remove the outside cap of the isolator and IMMEDIATELY deliver sterilant all along the inner cap/isolator port juncture WITHOUT previously trying to remove any bedding or debris from that area. Capillary action will then pull the sterilant into the space between the lip of the inner cap and the port. It is absolutely imperative that one NOT touch the inner cap at this time.

Nota Bene: Some inexperienced technicians take a paper towel and push up against the inner cap, unknowingly creating a temporary “privileged site” as they wisk away bedding and then release the pressure against the inner cap.

- 2) If bedding or debris is in that area, it will be sterile or will only contain what which is already inside the isolator. It can be removed AFTER the contact time is up following the closing of the outer cap, and the inner cap is opened.

Deliver sterilant to the entire inside of the isolator port by spraying or liberal swabbing with a small handle mop.

Thoroughly deliver a copious amount of sterilant to the outside surfaces of the TD by your method of choice (spraying, swabbing, etc.).

Place the wetted TD(s) into the port.

Deliver copious amounts of sterilant to the inside of the outer cap, including the lip, with or without a nipple(s). Some outside caps have 1, 2 or no nipples at all.

If the outer cap has a nipple(s), then place the cap onto the port and spray sterilant into the nipple until the inside of the port is saturated. Then spray the rubber stopper(s) with sterilant and quickly push them into the nipple(s). If the outside cap has no nipples, then put the wetted cap onto the port leaving a small flap at the top so that one can spray sterilant into the port just before closing the flap. After the cap has been fully put on, secure it in place with either tape (wipe the area dry with paper towels so the tape will adhere or use a clamp).

After the approved contact time, which can vary from sterilant to sterilant, open the inside isolator cap at the bottom and allow the sterilant to drain into a small container and then cap the container to only allow a minimum of sterilant to enter the isolator's atmosphere, unless one is using "activated hydrogen peroxide" as that sterilant only breaks down into oxygen and water.

Nota Bene: The first sterilant used, peracetic acid, is highly noxious and the inventor of this system of "cold sterilization (at room temp), Dr. Trexler, would always neutralize the sterilant through a nipple on the inside cap with a reducing agent like 1% sodium thiosulfate before removing the inside cap to protect the animals inside from exposure to its highly irritating fumes. Those laboratories that continue to use peracetic acid, no matter what concentration, should neutralize it before opening the inside cap.

Today, most laboratories use a chlorine dioxide based sterilant which does not require neutralization. Nevertheless, its concentration in the interior of the isolator should be kept to a minimum by capturing the residual liquid chlorine dioxide solution in a container that can then be sealed after it is drained from the bottom of the port after the inside cap is initially opened at the bottom. The inner cap should not have a nipple unless peracetic acid is used as the sterilant.

## **Introducing sterilized TDs into an isolator using an isolator supply cylinder and a transfer sleeve.**

After the autoclaved supply cylinder containing the TDs has been allowed to cool after autoclaving, proceed to attach the cylinder to the isolator port as is routinely done with all supply cylinders containing feed, bedding, swabs, etc. as follows:

Inspect the cylinder for any possible breaches of its integrity such as puncture wounds to the filter medium and/or Mylar™ film and if your facility used a chemical indicator of sterilization for each cylinder, then observe the result through the Mylar™ film and only proceed if the indicator demonstrates that the conditions for sterilization had been met.

Obtain an appropriate diameter connecting sleeve for the size of your port and supply cylinder and make sure it is clean to the naked eye.

The connecting sleeve should have two “nipples” that will allow the sterilant to be sprayed in after both ends have been secured.

Attach one end of the connecting sleeve to the supply cylinder after wetting down that end of the cylinder with sterilant as well as the inside of that end of the connecting sleeve that is going to be placed over the outside of the cylinder. Pull the end of the sleeve over the end of the supply cylinder being careful not to puncture the Mylar™ film.

Dry off any excess sterilant before applying the tape (at least two wraps) or metal clamp apparatus.

Wet the remaining insides of the connecting sleeve and the Mylar™ film.

Prepare the isolator entry port as in steps 1 through 3 as described in Method A.

Wet the outside surface of the isolator port where the transfer sleeve will be placed and then place the open end of the transfer sleeve over that area. Wipe dry the

outside of the sleeve and port and tape the sleeve onto the port with at least 2 wraps. Alternatively, use a metal clamp to secure the end of the transfer sleeve to the port.

Spray sterilant through one nipple until it starts coming out off the second nipple and then take two rubber or plastic stoppers totally wet with sterilant, and pug the two two nipples with them.

Dry the outsides of the stoppers and nipples and then tape them together to prevent a plug from popping out.

Wait the appropriate contact time based on the sterilant used and then open the inside cap of the isolator and move the cylinder as close as possible to the entry port.

Puncture the Mylar™ with a forceps or similar tool and remove the Mylar™ to allow for the TDs to be moved from the cylinder and pass them through the transfer sleeve into the isolator. This is most conveniently done by having one person holding up the cylinder and the other retrieving the TDs pulling them into the isolator.

Replace the inside cap and secure it with rubber band(s).

Remove the top of the TD(s) and place as many animals into the disks as needed.

After the TDs are stocked, just remove the inner cap and then place them into the connecting sleeve and close the inner cap remembering to put on the rubber bands.

The connecting sleeve can then be taken off the port and the populated TDs removed.

If radiation is planned, it is recommended that no animals be radiated for at least two days to allow acclimation to normal homeostasis, so it is recommended that they should be transferred to MI cages for a couple of days

# **How to Introduce TDs into an Isolator and then transfer them into another Isolator.**

## **Introduction:**

This is accomplished by simply using a transfer sleeve attached to two isolator ports in which taped TDs “off the shelf” are wetted with sterilant and placed into the connecting sleeve. Then the TDs can be introduced into the “donor” isolator, animals stocked into the TDs, and then the populated TDs can be transferred into the “recipient” isolator via the transfer sleeve.

The procedures used to connect an isolator sleeve from the end of a supply cylinder are now simply used to connect the sleeve between two isolator ports. The ports of both isolators are prepared as described in Method A, steps 1 through 3, repeated here:

Remove the outside cap of the isolator and IMMEDIATELY deliver sterilant all along the inner cap/isolator port juncture WITHOUT previously trying to remove any bedding or debris from that area. Capillary action will then pull the sterilant into the space between the lip of the inner cap and the port. It is imperative that one NOT touch the inner cap at this time.

Nota Bene: Some inexperienced newcomers take a paper towel and push up against the inner cap, unknowingly creating a temporary “privileged site” as they wisk away bedding and then release the pressure against the inner cap.

If bedding or debris is in that area, it will be sterile or will only contain what which is already inside the isolator. It can be removed AFTER the contact time is up following the closing of the outer cap, and the inner cap is opened.

Deliver sterilant to the entire inside of the isolator port by spraying or liberal swabbing with a small handled mop.

The connecting sleeve can then be attached as described in Method B to the one of the two isolator ports and then as many TDs taped shut and wetted with sterilant

as desired can be entered into the wetted sleeve, after which the other end of the sleeve can be attached to the other isolator port and the final spray of sterilant can be delivered through the connecting sleeves nipples. As usual, the stoppers for the nipples should then be wetted with sterilant, inserted into the nipples, the outside area dried and then the stoppers tapped to the nipples to keep them secured.

After the appropriate contact time, the TDs can be brought into the donor isolator, the cap closed, and the TDs populated. Then the inner cap of the donor isolator can be reopened and the stocked TDs can be placed into the connecting sleeve, the inner cap of the donor isolator closed and finally the inner cap of the recipient isolator can be opened and the TDs brought in.

# **How to Introduce empty TDs into an Isolator, populate them and then transfer the animals into Microisolation caging**

## **Introduction:**

Sterilized and taped TDs “off the shelf” can be introduced into an isolator, stocked and then the animals removed for allocation to microisolation housing simply by using what is known as a “dummy” supply cylinder.

This is merely the solid end of a supply cylinder at the opposite end of where the Mylar™ film would normally be added. Of course, any comparable nonporous, smooth-edged piece of metal or plastic could also be used.

Attach the “dummy” end of the supply cylinder to the port of the isolator via a connecting sleeve as outlined in Method B and placing the desired number of taped shut TDs inside the connecting sleeve before attaching it to the isolator.

Then bring in the TDs, stock them, replace them in the connecting sleeve, close the inside cap of the isolator, detach the sleeve, and remove the populated TDs. Then remove the tape from the ventilation holes and take the TDs to the hood that will be used in transferring the animals into microisolation caging. Transfer the animals into MI cages not exceeding the appropriate density allowable by the NIH guidelines.