

Protocol for importation of water supplies after setting up an isolator

To bring water into the isolator, 1000ml plastic bottles need to be autoclaved within the transport drum and following this an aseptic connection needs to be made with the isolator in question. **The preparation of the water bottles prior to putting them into the autoclave is critical – they must not be overfilled beyond 900ml in a 1000ml bottle, the bottle tops must be loose and the autoclave program must not be interrupted. The consequences of ignoring these could be a build up of pressure in one of the water bottles causing it to explode inside the transport drum.**

Materials for first transport cylinder connection

A. Inside the drum

1. 15 1000ml Ancare plastic bottles¹
2. 1 rack for the bottles
3. 3x 100ml Duran flasks with gauze swabs inside contained within bottle holder each marked with serial number²
4. Baxter water
5. 121°C chemical indicator strip

B. Additional materials required

1. Transport drum and second to bottom tray as base
2. 45cm wide aluminum foil
3. 3 absorbent pads
4. Mylar film
5. Vinyl tape³
6. Free autoclave time (make reservation ahead of time)
7. Spray gun and hose
8. Spray gun container with 100ml of 2% peracetic acid (freshly made up). The lid on the container should be tightly screwed down.
9. Swab loaded into swab holder
10. 250ml Duran flask to hold 10ml of 2% peracetic acid (freshly made up).
11. Access to electronic form for cylinder connection (GnotoformABC.xls, where ABC is the three-digit isolator code⁴—see **Gnoto1010** for codes).

Procedure for importation of water supplies into an isolator

1. Clean out the transport drum and remove any old tape.

¹ Jorum will make up codes for a series of sample bottles; one of these coded bottles should be included with bottles that will be imported into the isolator.

² Coding for the sample bottles. These are in groups of 6 and **coded in the standard fashion** with sample labels (format is 020520051 where the tube was prepared on 02 of May (05) Year (2005) and number (1);

³ **Colour Codes for Vinyl Tape**

Red – Cages & Equipment

Blue – Water

Green – Bedding & Food

⁴ For example, Gnotoform101.xls refers to isolator 101, Gnotoform102.xls refers to isolator 102, and so on.

2. Put tray #5 into the drum to provide a platform for the materials.
3. Prepare the water bottles by filling each bottle with **900ml** of Baxter water. Place a small piece of autoclave tape on the front of each bottle and **make sure that the cap is loosely on – screw until it is just firm and then back it off 1/2 of a turn (this is to ensure that pressure does not build up in the bottles during the autoclaving process)**. Put the bottles in the basket.
4. Tear off a piece of 45cm wide aluminum foil that is slightly longer than the drum.
5. Lay the aluminum foil in the base of the drum so that it reaches all the way to the back of the drum. Fold the excess up in the front end. On the sides, tuck the foil under where shelf #5 sits. Try not to tear the foil.
6. Fold two absorbent pads into halves and place on the aluminum foil in the base of the drum to catch any overflow. Place a third folded absorbent pad at the very front of the drum to absorb pooled water.
7. Put the basket of water bottles onto the tray.
8. Load the drum with the other materials in List A above.
9. Seal the drum with Mylar film as per **Gnoto1023**.
10. Autoclave the drum on appropriate program (**see Gnoto1011**). **Under no circumstances should the autoclave program be interrupted, as this may lead to an explosion of one of the water bottles in the autoclave.**
11. Remove the drum from the autoclave and bring into the gnotobiotic unit (**see Gnoto1007**).
12. Remove only the outer quadralock door of the isolator.
13. Obtain a clean sleeve and seal one end to the drum and the other to the quadralock by passing over the rubber ring and sealing down with the adjustable clamp.
14. Connect drum to isolator as per **Gnoto1024**.
15. Import 14 water bottles, screw down the tops tight and arrange them neatly.
16. Keep one water bottle after screwing down the top tight for sending to the lab for sterility assays.
17. Take samples in the isolator for bacterial culture. One bottle should remain unopened. The remaining two bottles should be opened and 6-7 samples of faeces from random cages should be transferred into each bottle using the long forceps in the isolator. The sample bottles are then transferred back to the transport cylinder.
18. Close inner quadralock door.
19. Detach the sleeve and place it to be cleaned prior to next use.
20. Complete GnotoformABC.xls online, print off a copy, and file it in the appropriate file for the isolator.
21. Place the sample bottles in the tray in the corridor.
22. Store the transport cylinder.