MilliporeSigma

User Guide

Steritest® Devices

TZHALA2--
TZHVAB2--
TZHVSL210
TZHAPC210
TZH-CA210
TZH-LV2--
TZH-SV2--

for

- **Liquids in Ampoules and Collapsible Bags**
- **Liquids in Plastic Containers and Cartridges**
- **Antibiotics**
- **Solvents, Creams, Ointments, and Veterinary Injectables**
- **Liquids in Large Vials**
- **Liquids in Small Vials**

The life science business of
Merck KGaA, Darmstadt, Germany
operates as MilliporeSigma in
the US and Canada.

www.sigmaaldrich.com

Millipore®

Contents

Introduction 3
Operator and Equipment Safety 4
Outline of Catalog Numbers and Steritest® Devices Applications 5
Specifications and Operating Requirements 9
    Pump Speed Recommendations 10
Product information 11
    Product information on the box label 11
    Product information on the blister cover sheet 11
Steritest® Device Components 12
Using the Steritest® Device for Sterility Testing 13
    Before using the Steritest® Device 13
    Installing the Steritest® Device 13
    Pre-Wetting the Membranes 15
    Testing the Product 18
    Rinsing the Tubing, Canisters, and Membranes 26
    Adding Culture Media to the Canisters 27
    Culturing the Media 29
    Reading the Canisters 31
Troubleshooting 32
Accessories for Sterility Testing 34
Standard Product Warranty 35
Technical Assistance 36

Introduction

The Steritest® device offers a closed method for sterility testing of pharmaceutical products in a wide range of packaging configurations.

The Steritest® device requires no open transfer of the sample, minimizing the risk of false positive results from adventitious contamination.

A special peristaltic pump (Steritest® Symbio or Equinox pump) transfers product directly from its container through sterile tubing into the sealed Steritest® filtration chambers.

Many types of sterile products can be tested with the Steritest® device, including:

- Large- and small-volume parenteral fluids in glass or plastic bottles
- Liquids in ampoules, collapsible bags, and plastic containers
- Antibiotics (liquids or reconstituted powders in vials)
- Solvents, creams, ointments, and veterinary injectables
- Liquids in cartridges
- Liquids in large or small vials

Any microorganisms present in the sample are captured on the microporous membrane in the Steritest® canisters. Appropriate culture medium is pumped into each canister separately to promote the growth of the captured organisms. The canisters are incubated and examined for contamination in accordance with the relevant pharmacopoeia: United States (USP), Europe (EP), and Japan (JP).

This manual details the procedure for the installation and use of several Steritest® devices with the Steritest® Symbio or Equinox pump.

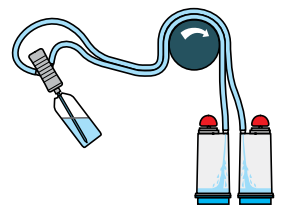
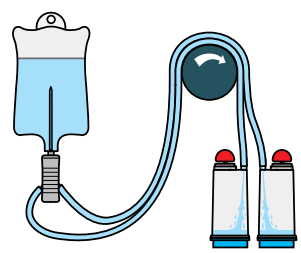
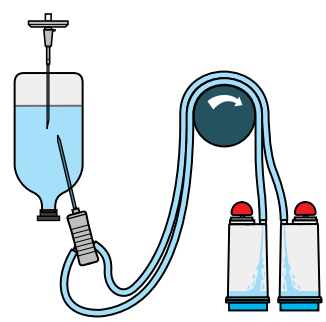
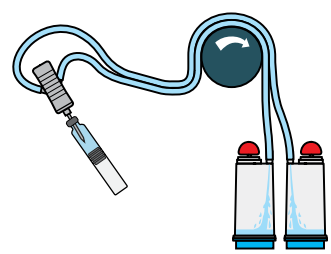
Operator and Equipment Safety

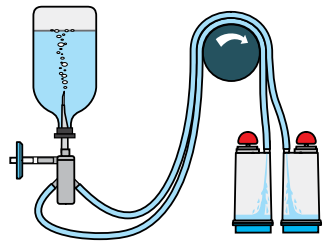
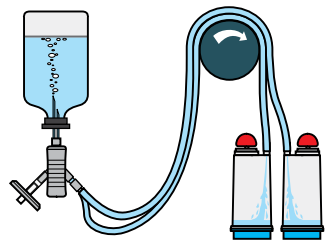
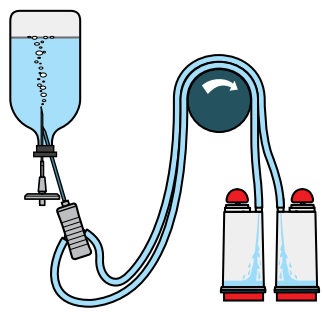
Everyone who will operate or be near the Steritest® system must comply with the following:

- Read and understand the **Steritest® Symbio Pump User Guide** (see Accessories for Sterility Testing) and this user guide before beginning the sterility test. Failure to follow operating instructions could result in user injury or inaccurate sterility testing results.
- Any alteration of the device from factory specifications may cause unsafe conditions and will void the product warranty.
- Any attempt to use the device in a manner not specified in this user guide will void the product warranty and may result in damage to the sterility test and operator injury.
- Never expose the equipment to extreme temperatures. Operating temperature must be between 15 °C and 45 °C (59 and 113 °F).
- Use the Steritest® devices only with the Steritest® Symbio or Equinox pump and accessories.
- Use only accessories designed for the pump (see Accessories for Sterility Testing). Using accessories not designed for the pump could result in user injury or damage to the instrument.
- When filtering hazardous liquids, wear and use proper protective clothing and equipment for the handling and the disposal of the liquid being tested.
- In case of contact with the tested product, refer to the safety datasheet of the product for first aid measures.
- Dispose the filtrate in accordance with local regulations.
- Do not use the Steritest® Symbio or Equinox pump and devices to filter flammable products.
- Handle the Steritest® needle and the Velax® cutting clamp with care to prevent injury.

Outline of Catalog Numbers and Steritest® Devices Applications

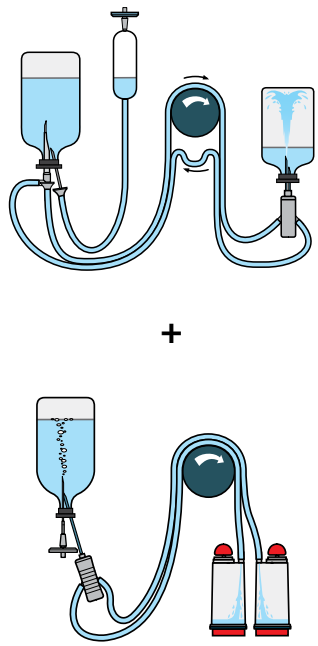
NOTE The Steritest® devices are packaged ten to a box.

Device Application	Needles Set	Catalog Number	Illustration
For Products Without Antimicrobial Agents Blue canister base indicates mixed esters of cellulose membrane. This membrane provides an optimal filtration flow rate for standard products.			
Steritest® device **for liquids in ampoules and collapsible bags**	• Single needle for easy access to ampoules or collapsible bags • Separate vent needle	TZHALA210 TZHALA205 (double packed)	 
Steritest® device **for liquids in plastic containers**	• Single needle minimizing coring when piercing plastic containers • Separate vent needle	TZHAPC210	
Steritest® device **for liquids in cartridges**	• Single needle for easy access to cartridges (20 mm length) • Separate vent needle	TZHACA210	

Steritest® device **for liquids in large vials**	Vented double needle for large vials with septa	TZHALV210 TZHALV205 (double packed)	
Steritest® device **for liquids in small vials**	Vented double needle for small vials with septa	TZHASV210 TZHASV205 (double packed)	
For Products with Antimicrobial Agents Red canister base indicates low-adsorption Durapore® PVDF (polyvinylidene fluoride) membrane and specific drain design. This optimizes the rinsing of products that inhibit microbial growth.			
Steritest® device **for antibiotics** (liquids)	• Single needle and connections designed to reduce the risk of antimicrobial residuals • Separate vent needle • Recommended Accessories: Sterile vent needles for safe transfer of rinse fluid and liquid media: TEFG02525 (*)	TZHVAB210 TZHVAB205 (double packed)	

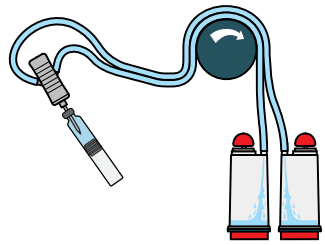
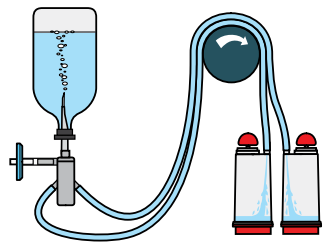
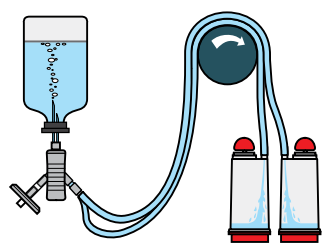
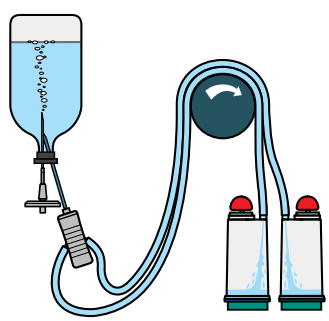
<table>
<tr>
<td rowspan="2">Steritest® device
for antibiotics (powders and superpotent antibiotics)
Steridilutor® device
• Aseptically connects the diluent or dissolution fluid to the product container for dilution.
• Used for pooling superpotent antibiotics to reduce product-membrane contact time during the filtration.
• Diluted product subsequently filtered with Steritest® device TZHVAB2--

Use this device after pooling super potent antibiotics and/or after diluting powders</td>
<td>Steridilutor® device:
Tubing and needle assembly for antibiotics and products containing antimicrobial activity that require dilution or dissolution.

Steritest® device TZHVAB2--:
• Single needle and connections designed to reduce the risk of antimicrobial residuals
• Separate vent needle</td>
<td rowspan="2">Steridilutor® Device (**)
+
TZHVAB210
TZHVAB205
(double packed)</td>
<td rowspan="2">  </td>
</tr>
<tr>
<td>Recommended Accessories:
Sterile vent needles for safe transfer of rinse fluid and liquid media: TEFG02525 (*)</td>
</tr>
</table>

* When testing antibiotics, it is recommended to not use the same vented needle for both product filtration and membrane rinsing / media transfer.

** If superpotent antibiotics or antibiotics in powder form are tested, the recommendation is to use Steridilutor™ Device for Vials (see Accessories for Sterility Testing: catalog number TZV000010 without expansion chamber or TZVC00010 with expansion chamber) to pool and/or predissolve antibiotics.

Steritest® device **for liquids in cartridges**	• Single needle for easy access to cartridges (20 mm length) • Separate vent needle	TZHVCA210	
Steritest® device **for liquids in large vials**	Vented double needle for large volume containers with septa	TZHVLV210 TZHVLV205 (double packed)	
Steritest® device **for liquids in small vials**	Vented double needle for small vials with septa	TZHVSV210 TZHVSV205 (double packed)	
For Products that Require Increased Chemical Compatibility Green canister base indicates low-adsorption Durapore® membrane, specific drain design, and polyamide canister polymer. The canister is designed for testing products dissolved in solvents such as IPM (isopropyl myristate). Canister connections and reinforced base structure provide better resistance to pressure. These features optimize the chemical compatibility to chemically aggressive products and products dissolved in solvents.			
Steritest® device **for Solvents, Creams, Ointments, and Veterinary Injectables**	• Single needle for products in vials or ampoules • Separate vent needle	TZHVSL210	

Specifications and Operating Requirements

Materials of construction	Blister	PETG	
	Blister cover sheet	Tyvek®	
	Filtration chamber (canister)	Red or blue base devices	Acrylonitrile styrene (SAN)
		Green base devices	Polyamide 6-6 (nylon)
	Double lumen tubing with pump head placement marks	PVC	
	Needle(s)	Stainless steel and polyamide 6-6	
	Needle adapter	Polyamide 6-6	
	Canister base membrane	Blue base devices	Mixed esters of cellulose (HA) membrane, 0.45 µm
		Red or green base devices	Low-adsorption Durapore® (HV) membrane, 0.45 µm hydrophilic PVDF
	Canister vent filter	Durapore® membrane, hydrophobic	
Dimensions and Weight	Canister height	120 mm (4.7 in.)	
	Canister diameter	51 mm (2.0 in.)	
	Double tubing length	850 mm (33.5 in.)	
	Total weight (canister with 100 mL of culture medium)	145 g (0.32 lbs.)	

Operating Requirements	Maximum temperature	45 °C (113 °F)
	Maximum operating pressure	3.65 bars at 25 °C (53 psi at 77 °F)
	Minimum flow rate (for water)	300 mL/min at 690 mbar (10 psi)

Pump Speed Recommendations

The following table indicates the recommended maximum pump speed to ensure:

- Excellent separation of the fluids between the two canisters for pre-wetting, rinsing, and test steps.

  NOTE These speeds have been established using purified water and may not be applicable to other fluids.

- Excellent culture media transfer in a canister.

	Catalog Number	Recommended Maximum Pump Speed			
		Pre-wetting/ Rinsing	Test	Culture Media Transfer	
				FTM	TSB
Blue canister base	TZHALA2xx	50	75	30	75
	TZHAPC210		75		
	TZHACA210		75		
	TZHALV2xx		150		
	TZHASV2xx		75		
Red canister base	TZHVAB2xx		75		
	TZHVCA210		75		
	TZHVLV2xx		150		
	TZHVSV2xx		75		
Green canister base	TZHVSL210		75		

Do not use a Steritest® device at a speed greater than the maximum recommended speed.

Filter pre-wetting and rinse fluids at speed 50 to ensure that the fluids flow through every pore of the membrane.

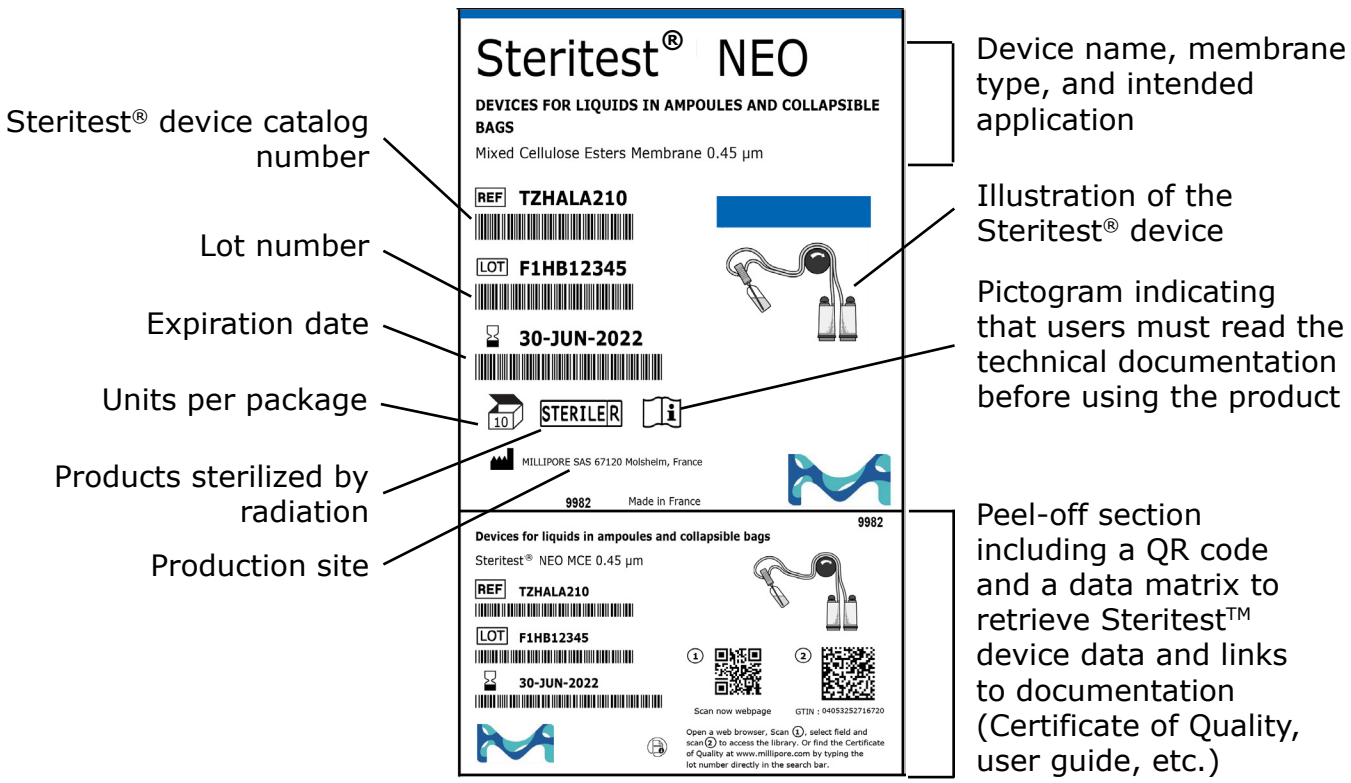
It is the responsibility of customers to establish the maximum pump speed applicable to their products and rinse fluids during the development of a new test method.

In most cases, filter the product tested for sterility as fast as possible to minimize the contact time between the product and the membrane. Do not exceed the maximum recommended speed. Ensure the product does not splash in the canisters. Splashing could prevent the removal of all product residue from the wall of the canister during the rinsing step.

Product information

Product information on the box label

Each box contains ten Steritest® devices and is identified by a two-part label. This is an example of the label for the Steritest® device TZHALA210.



Product information on the blister cover sheet

The information indicated on the box label and the data matrix are also printed on the blister cover sheet on each Steritest® device. This is an example of the Steritest® device TZHALA210 blister cover sheet.

Steritest® Device Components

The following components are common to all Steritest® devices specified in this user guide.

- Two canisters with clear graduation marking (100, 75, 50, and 25 mL marks)
- One double tubing with:
  - Colored tubing clamps (red and white) for discernible media filling and reduced risk for error
  - Pump head placement marks on tubing for optimized distribution of tubing on both sides of the pump head during installation
  - Velax® blue cutting clamp for disconnecting tubing without tools
- Needles set (not the same for all Steritest® devices)

  NOTE The vent needle can be either separate from the sampling needle or part of the sampling needle.
- An easy-to-open accessories bag that contains two red caps and two yellow plugs.

Using the Steritest® Device for Sterility Testing

Sanitize all consumables required for the sterility test (fluids, samples, Steritest® units, and so forth) before introducing them into the testing environment (laminar flow hood, biosafety cabinet, clean room, or isolator).

Remove all plastic covers from bottles of fluids or other containers. Decontaminate each septum with a nonwoven, lint-free cloth moistened with a disinfecting agent (sterile 70% isopropyl alcohol [IPA]).

If using double packed Steritest® devices, open the external easy-to-open plastic bag and remove the Steritest® blisters just before introducing them into any testing environment (laminar flow hood, biosafety cabinet, clean room or isolator).

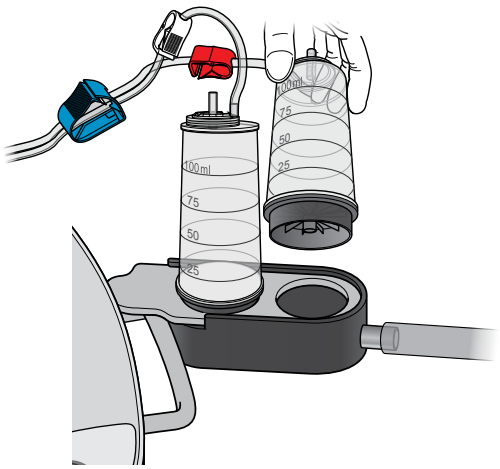
Before using the Steritest® Device

- Set up and switch on the pump. Refer to the **Steritest® Symbio Pumps User Guide** (see Accessories for Sterility Testing).
- Ensure that the pump drain tubing is connected to a liquid waste receptacle

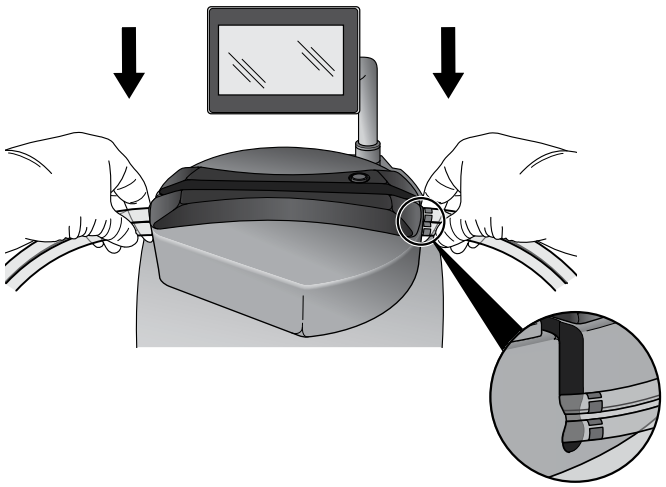
  NOTE Using the footswitch can facilitate the steps of starting and stopping the pump.

Installing the Steritest® Device

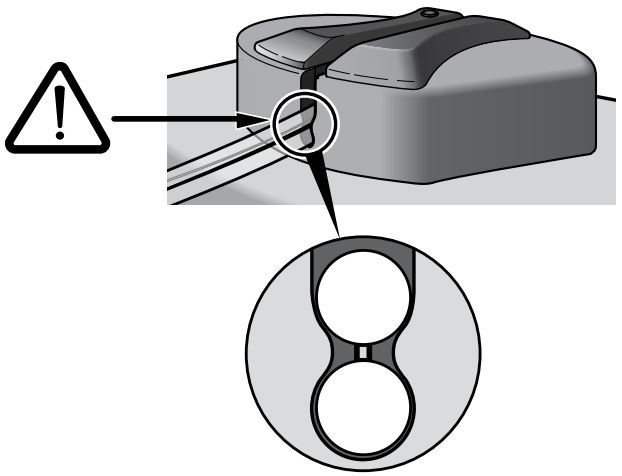
1. Open the Steritest® device package by peeling the blister cover sheet.
2. Remove the canisters and tubing needle set from the blister.
3. Place the two canisters upright on the drain tray and ensure that the Velax® cutting clamp is positioned on the tubing between the canisters and the pump head.



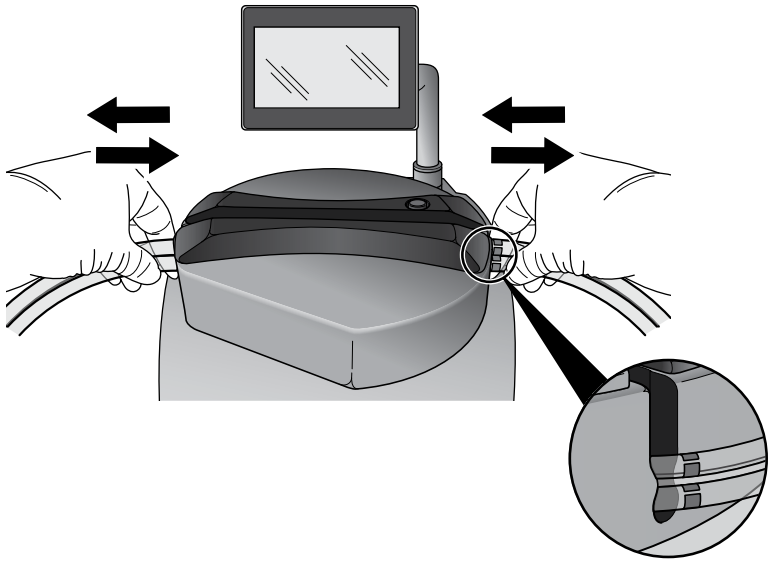
4. Install the tubing in the pump head by gently pulling the tubing and engaging it fully.



5. Ensure that the tubing is positioned on either side of the bosses on the right and left sides of the cover.



6. Slide the tubing back and forth to ensure that it is correctly positioned. Ensure that the gray marks on the tubing, on the right side of the pump head, are visible and very close to the pump head (optimized distribution of the tubing on both sides of the pump head).



NOTE If the tubing does not slide freely, remove and reinstall it.

7. Select either Standard Mode or Test Method Mode by pressing the control knob on the pump.
8. Close the pump head by pressing the  button.
9. Open the accessories bag and place the red caps and yellow plugs inside the blister package or on a decontaminated surface.

Pre-Wetting the Membranes

Pre-wet the membrane to minimize product adsorption and to facilitate filtration.

NOTES

Pre-wetting is recommended when testing samples like antibiotics, products containing preservatives, and all substances that have had an inhibitory effect on microbial growth during method development.

Establish the necessity and efficiency of the pre-wetting step during method development and prove it during validation.

Refer to the relevant pharmacopeia for rinse fluid recommendation.

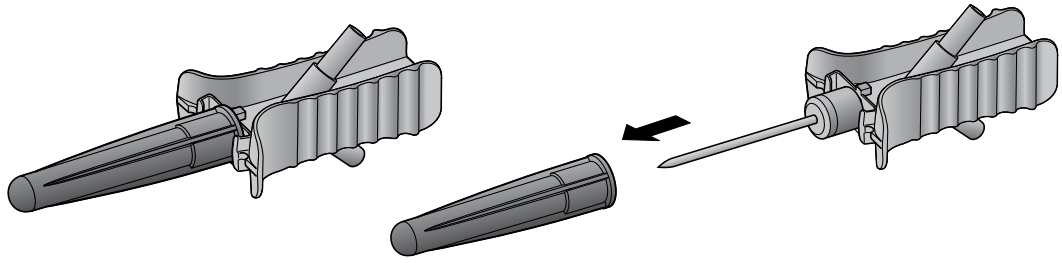
Inserting the Needle(s)

Insert the needle(s) into the pre-wetting fluid bottle septum while keeping the bottle upright on the workbench.

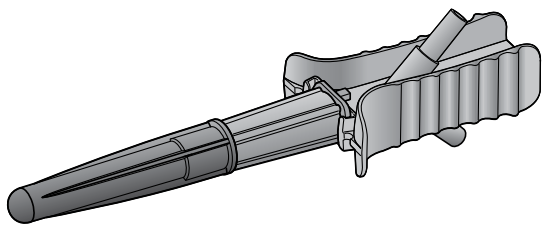
Using a Steritest® Device with a Separate Vent Needle

1. Remove the protective cap from the Steritest® separate vent needle and insert this needle fully into the septum of the pre-wetting fluid bottle.
2. Remove the protective cap(s) from the Steritest® single needle.

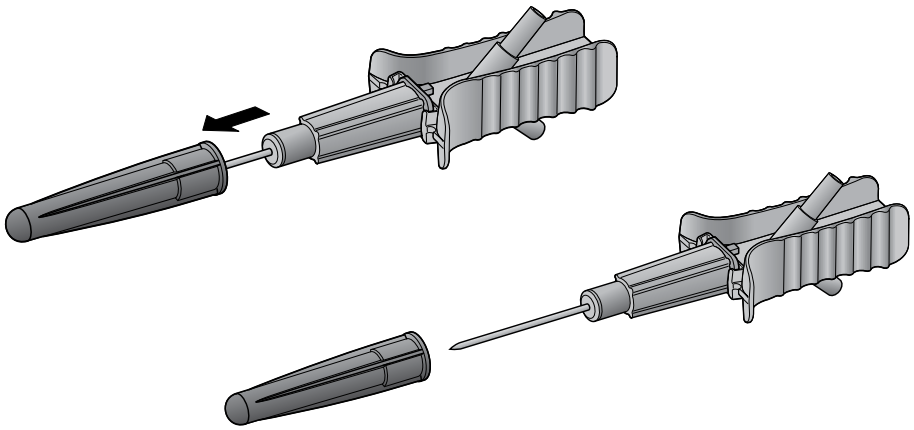
   The TZHxCA210 Steritest® NEO device is equipped with a short, 20-mm-long sampling needle protected by a dark-blue, one-piece plastic cap. Pull off the cap to expose the needle.



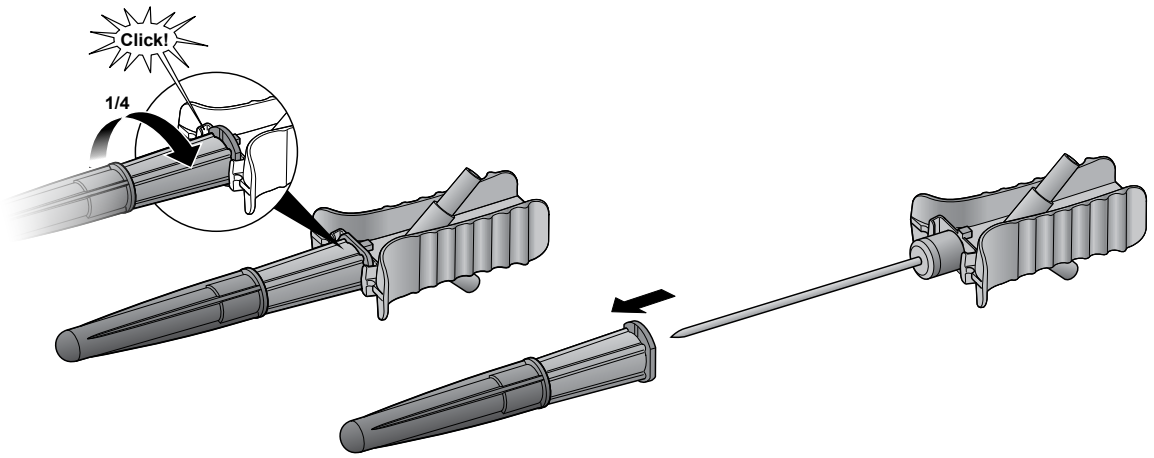
Other Steritest® NEO devices described in this user guide are equipped with longer sampling needles that can be used as either 35-mm- or 60-mm-long needles. The protective caps consist of two parts that enable users to select the needle length that will maximize safety depending on the containers to be sampled.



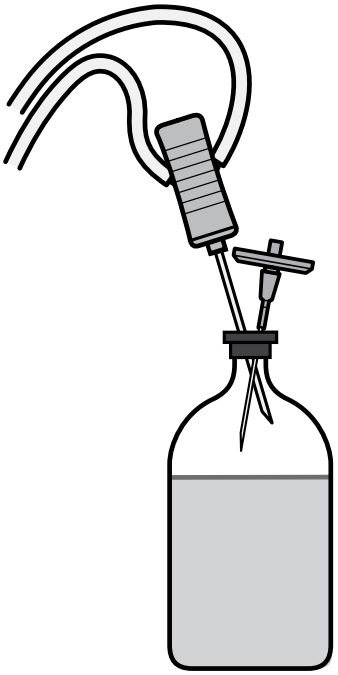
- To expose the 35 mm needle, pull off only the first clear or dark-blue short cap:



- To expose the 60 mm needle, twist the bottom part 1/4 of a turn and pull to remove the two parts of the cap together (bottom sleeve and short cap):

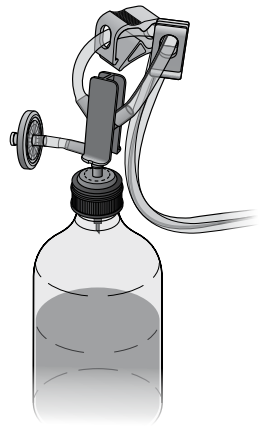


3. Insert the single needle into the pre-wetting fluid bottle septum but not as deep as the separate vent needle. This will prevent air aspiration in the product sampled.



Using a Steritest® Device with a Vented Needle

Remove the protective cap from the Steritest® vented needle and insert it fully into the septum of the 100 mL pre-wetting fluid bottle.



Pre-Wetting

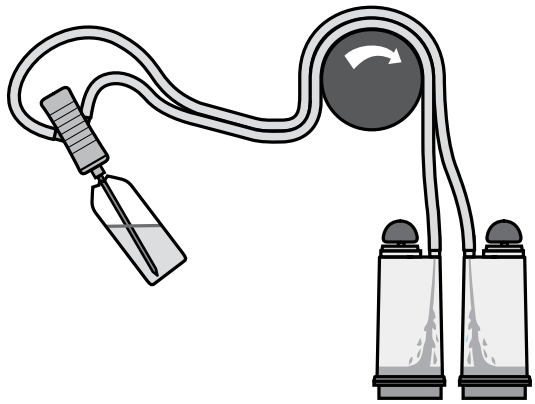
1. Set the pump speed at the appropriate value for the pre-wetting fluid characteristics (see Pump Speed Recommendations).
2. Start the pump.
3. After few seconds, turn the pre-wetting fluid bottle upside down and place it in the bottle holder so that the fluid will pre-wet the filter membranes.
4. Transfer approximately 50 mL of sterile pre-wetting fluid into each Steritest® canister.
5. Remove the pre-wetting fluid bottle from the bottle holder and place it upright on the workbench.
6. Place a red cap onto each canister vent to allow the pre-wetting fluid to start filtering through the membranes.
7. Remove the red cap from the top of each canister when there is approximately 25 mL of liquid in each canister (the level is indicated on the side of the canister). This ensures that the tested product is mixed with pre-wetting fluid when starting the filtration. This often improves the filtration of the product.
8. Stop the pump.
9. Reposition the red cap on the vent of each canister.

Testing the Product

Liquids in Ampoules

These instructions apply to the following Steritest® devices:

- TZHALA210
- TZHALA205
- TZHVAB210
- TZHVAB205
- TZHVSL210



NOTE The separate vent needle is not necessary when sampling liquids in ampoules.

1. Keep the vent needle inserted in the pre-wetting fluid bottle septum, and keep the bottle upright on the workbench.
2. Set the pump at a low speed to ensure the pump can be easily stopped before air is aspirated.

   NOTE Aspirating air from the test environment can cause contamination and might impair the correct splitting of the product between the two canisters.
3. Open the ampoule to be tested.

   NOTE Use the Steritest® ampoules breaker (see Accessories for Sterility Testing) to break the neck of the ampoules.
4. Remove the single needle from the pre-wetting fluid bottle septum.
5. Insert this single needle while holding the ampoule at a 45° angle.
6. Start the pump.

   NOTE The footswitch can be used to facilitate ampoule testing.
7. Pump slowly to allow the liquid in the ampoule to transfer first to the tubing and then to the canisters.

8. To prevent air from being drawn into the tubing, stop the pump when only the tip of the needle is still submerged in the liquid at the bottom of the ampoule.

   NOTE The pump timer can be used to secure this step.

9. Remove the needle.
10. Discard the empty ampoule.
11. For each additional ampoule to be tested, open the ampoule and repeat steps 5 to 10.
12. Remove the single needle from the last ampoule and insert it through the septum of the already vented bottle of pre-wetting fluid.

    NOTE Keep the bottle upright on the workbench.

13. Start the pump.
14. Run the pump until all the liquid that remains in the tubing has been transferred to the canisters and filtered through the membranes.

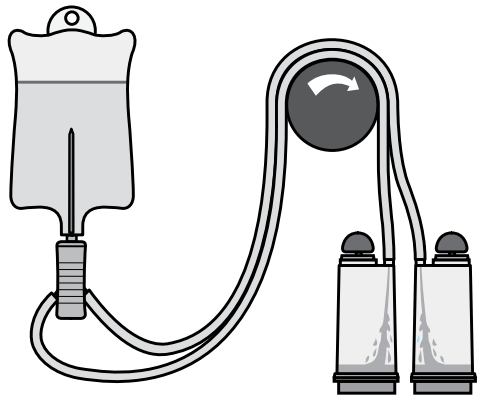
    NOTE Small liquid drops may remain in the tubing.

15. Stop the pump.

Liquids in Collapsible Bags

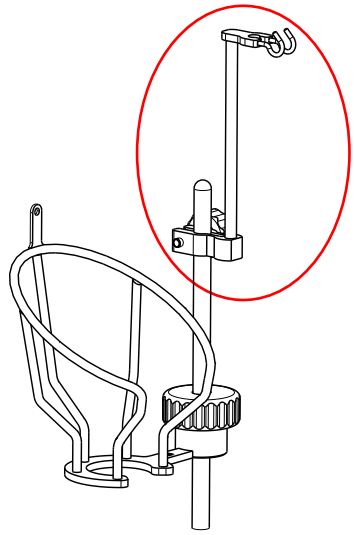
These instructions apply to the following Steritest® devices:

- TZHALA210
- TZHALA205
- TZHVAB210
- TZHVAB205
- TZHVSL210



NOTE The vent needle is not necessary when sampling product from collapsible bags. Let it remain in the pre-wetting fluid bottle septum.

1. Set the pump speed at the appropriate value according to the product characteristics (maximum pump speed 75).
2. Invert the collapsible bag and hang it on the Steritest® holder for sterile bags (see Accessories for Sterility Testing) that has been mounted on the bottle support rod.



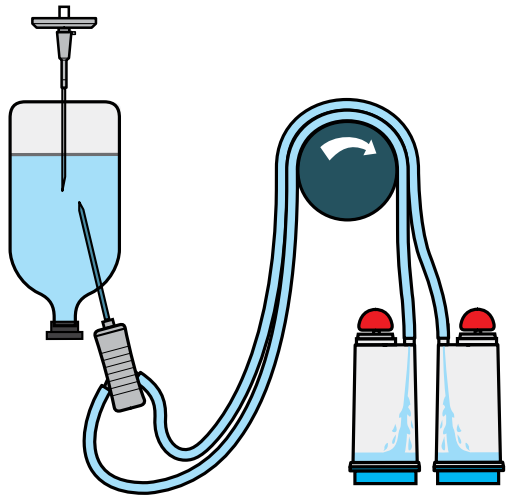
3. Remove the single needle from the pre-wetting fluid bottle septum.
4. Insert this needle through the septum of the collapsible bag.

   NOTE Ensure that the needle tip reaches the inside of the bag.
5. Start the pump.
6. Stop the pump when the necessary volume of product has been withdrawn from the bag.
7. Remove the bag from the Steritest® holder and put it on the workbench.
8. Remove the needle.
9. For each additional bag to be tested, repeat steps 4 to 8.
10. Insert the single needle removed from the last bag tested into an available sterile vented container (for example, the pre-wetting fluid bottle septum).
11. Start the pump.
12. Run the pump until all the liquid is transferred to the canisters and filtered through the membranes.

    NOTE Small liquid drops may remain in the tubing.
13. Stop the pump.

Liquids in Plastic Containers

These instructions apply to the following Steritest® device: TZHAPC210



1. Set the pump speed at the appropriate value according to the product characteristics (maximum pump speed 75).
2. Remove the separate vent needle from the pre-wetting fluid bottle septum.
3. Invert the plastic container. Pierce the bottom of the container with the vent needle.
4. Remove the single needle from the pre-wetting fluid bottle septum or from the plastic container that has already been tested.
5. Pierce the container close to the container neck with the sampling needle. If suitable, place the container in the bottle holder.
6. Start the pump.
7. Stop the pump when the necessary volume of product has been withdrawn from the plastic container.
8. For each additional plastic container to be tested, remove a separate vent needle from the container tested and repeat steps 3 to 7.

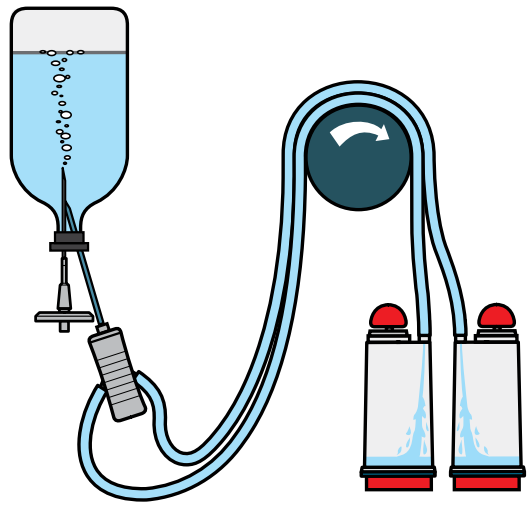
NOTE After sampling the last container, run the pump until all the liquid in the container is transferred to the canisters and filtered through the membranes. Small liquid drops may remain in the tubing.

Antibiotics

These instructions apply to the following Steritest® devices:

- TZHVAB210
- TZHVAB205

NOTE Combine the content of small antibiotics containers into one single bottle to reduce product-membrane contact time during the sample filtration.



1. Set the pump speed at the appropriate value according to the product characteristics.

   NOTES

   Use the highest possible pump speed up to 75 to reduce the contact time between the antibiotics and the membrane.

   Prevent splashing; it is difficult to rinse out droplets of product at the top of the wall of the canister.

2. Remove the separate vent needle from the pre-wetting fluid bottle septum and insert it fully into the product bottle septum while keeping the product bottle upright on the workbench.
3. Remove the single needle from the pre-wetting fluid bottle septum and insert it into the product bottle septum but not as deep as the separate vent needle.
4. Start the pump.
5. After few seconds, turn the product bottle upside down and place it in the bottle holder.
6. Stop the pump when the entire contents have been transferred to the canisters.

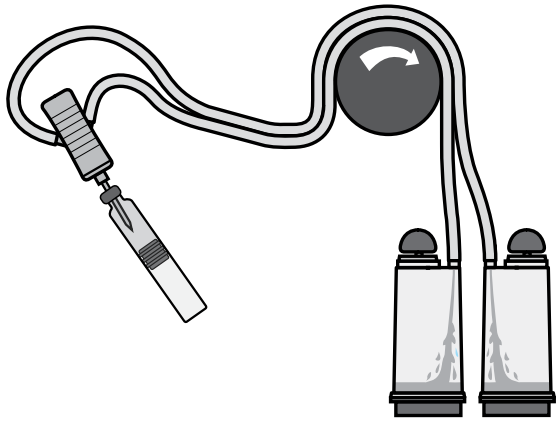
   NOTE Small liquid drops may remain in the tubing.

7. Remove the bottle from the bottle holder and place it upright on the workbench.

Liquids in Cartridges

These instructions apply to the following Steritest® devices:

- TZHACA210
- TZHVCA210



NOTE The separate vent needle is not necessary for sampling liquids in cartridges.

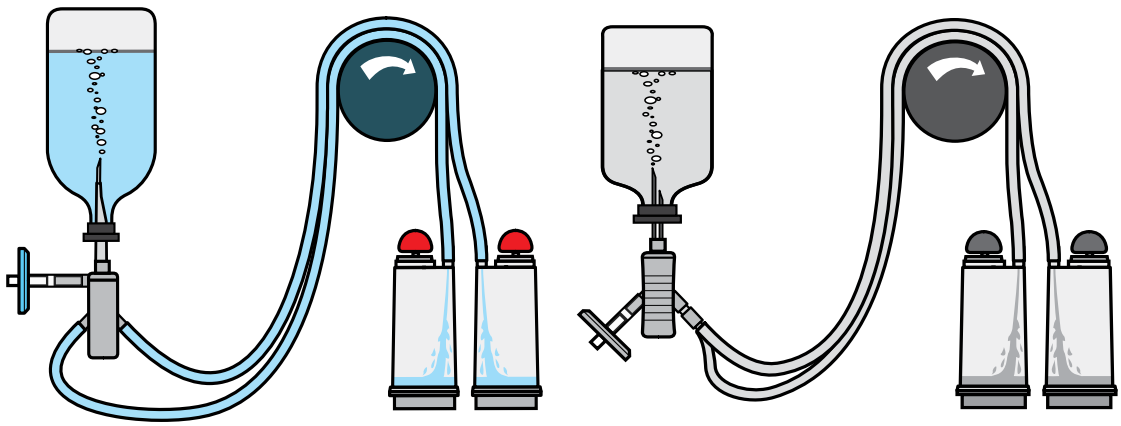
1. Keep the vent needle inserted in the pre-wetting fluid bottle septum and keep the bottle upright on the workbench.
2. Set the pump speed at the appropriate value according to the product characteristics and volume (maximum pump speed 75).
3. Remove the single needle from the pre-wetting fluid bottle septum.
4. Insert this single needle through the septum of the cartridge to be sampled.
5. Start the pump.
6. Pump to allow the liquid in the cartridge to transfer first to the tubing.
7. Remove the needle.
8. Discard the empty cartridge.
9. For each additional cartridge to be tested, repeat steps 4 to 8.
10. Insert the single needle removed from the last cartridge sampled into the septum of the vented pre-wetting fluid bottle while keeping the bottle upright on the workbench.
11. Start the pump.
12. Run the pump until all the liquid remaining in the tubing is transferred to the canisters and filtered through the membranes.

    NOTE Small liquid drops may remain in the tubing.
13. Stop the pump.

Liquids in Large and Small Vials or in Bottles

These instructions apply to the following Steritest® devices:

- TZHALV210
- TZHALV205
- TZHVLV210
- TZHVLV205
- TZHASV210
- TZHASV205
- TZHVSV210
- TZHVSV205



1. Set the pump speed at the appropriate value for the pre-wetting fluid characteristics.
2. Remove the vented double needle from the pre-wetting fluid bottle septum.
3. Hold the product container to be tested upright on the workbench.
4. Insert the vented double needle fully into the septum of the sample container.
5. Start the pump.
6. After few seconds, invert the glass bottle or vial. If suitable, place it in the bottle holder.
7. Stop the pump when the necessary volume of product has been withdrawn and transferred to the canisters.

   NOTE If there is only one bottle or one vial to test or if it is the last bottle or vial to be tested, let the pump run until all the liquid has been transferred to the canisters and filtered through the membranes.
8. Remove the product container from the bottle holder and place it upright on the workbench.
9. For each additional bottle or vial to be tested, repeat steps 3 to 8.

Rinsing the Tubing, Canisters, and Membranes

When testing solvents or using isopropyl myristate (IPM), do not rinse with aqueous fluids. Rinse with sterile solvents (like IPM).

For other applications, perform the rinsing procedure using sterile rinse fluid.

Consult the relevant pharmacopeia for recommended formulae.

Determine the volume of rinse fluid during the method development procedure.

CAUTION

If Fluid K or IPM is used during the rinsing procedure, perform a last rinsing step with a product free of polysorbate 80 (such as fluid A) to minimize the risk of microbial growth inhibition.

1. Set the pump speed at the appropriate value according to the test method developed. Do not exceed the maximum speed for the Steritest® device being used.
2. Remove the red cap from each canister vent.
3. Remove the vent needle and/or the single needle or the vented needle adapter from the last sample container tested, if not already done.
4. Insert the separate vent needle into the rinsing fluid bottle septum (the bottle that is upright on the workbench). Then insert the single needle, but not as deep as the single needle.

   OR

   Fully insert the vented needle into the rinsing fluid bottle septum (the bottle that is upright on the workbench).

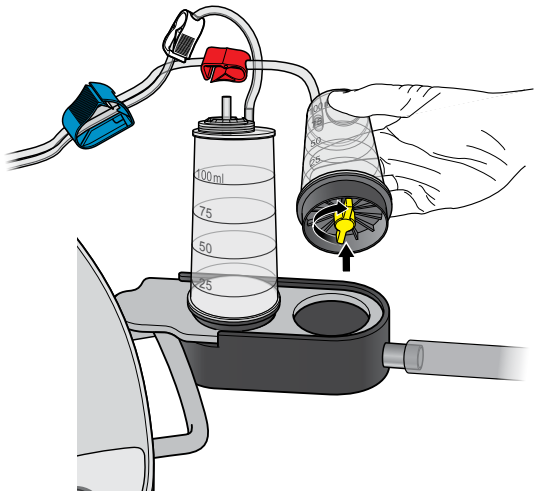
   NOTE If the product tested contains antibiotics, preservatives, or any other substances that could inhibit microbial growth, discard the vent needle used up to this step and replace it with a new one. This will prevent the risk of false negative results. Use a new sterile spare vent needle (see Accessories for Sterility Testing) for the remaining steps (rinsing the tubing, canisters, and membranes, and adding culture media to the canisters).
5. Start the pump.
6. After few seconds, turn the rinsing fluid bottle upside down and place it in the bottle holder.
7. Fill each canister with 100 mL of rinsing fluid.
8. Remove the rinsing fluid bottle from the bottle holder and place it upright on the workbench.
9. Run the pump until all the liquid remaining in the tubing is transferred to the canisters.
10. Stop the pump.
11. Put the red caps on the vents of the canisters.
12. Filter the entire rinsing fluid volume through the membrane of the canister.
13. Stop the pump.
14. Repeat steps 2 to 13 to continue rinsing (100 mL of rinse fluid at a time) per the validated standard operating procedure.

Adding Culture Media to the Canisters

Have 100 mL of sterile Fluid Thioglycollate Medium (FTM) and 100 mL of sterile Tryptic Soy Broth (TSB) ready and packaged in separate containers with septum caps.

1. Remove the red caps from the canister vent.
2. Lift one of the canisters from the drain tray and place a yellow plug onto the bottom outlet port of the canister.

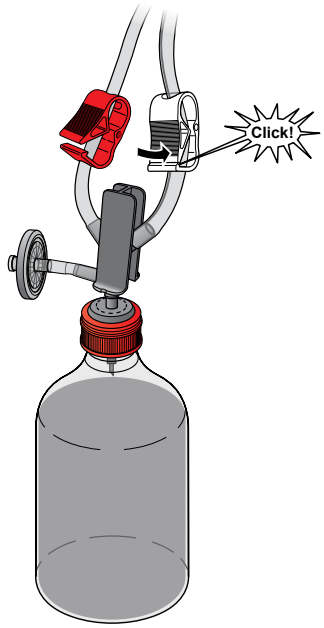
   NOTE Secure the yellow plug by twisting it 90° while pushing it in place on the bottom outlet port of the canister.



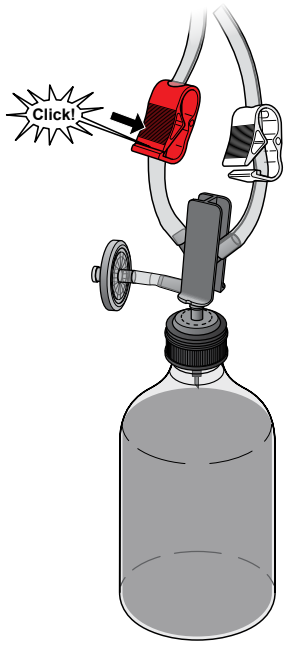
3. Return the canister to the drain tray.
4. Place a yellow plug onto the bottom outlet port of the second canister.
5. Pick up the FTM bottle and set the pump speed to low (30 maximum).

   NOTE Low pump speed minimizes medium oxygenation during the transfer of FTM.

6. Remove the Steritest® needle(s) from the last rinsing fluid bottle septum and insert it or them into the septum of the FTM bottle. If not using a vented needle, insert the separate vent needle deeper than the single needle.
7. Close the white clamp located on the needle side.



8. Start the pump.
9. After few seconds, turn the culture media bottle upside down and place it in the bottle holder so that the FTM transfers into one canister.
10. Stop the pump as soon as the culture media bottle is empty and the tubing is clear of media.
11. Remove the culture media bottle from the bottle support and place it upright on the workbench.
12. Remove the Steritest® needle(s) from the septum of the FTM bottle and insert it or them into the septum of the TSB bottle.
13. Open the white clamp.
14. Close the red clamp located on the needle side.



15. Increase the pump speed to medium speed (75 maximum).
16. Start the pump.
17. After few seconds, turn the culture media bottle upside down and place it in the bottle holder so that the TSB will transfer into the second canister.
18. Stop the pump as soon as the culture media bottle is empty and the tubing is clear of media.
19. Open the red clamp.

Culturing the Media

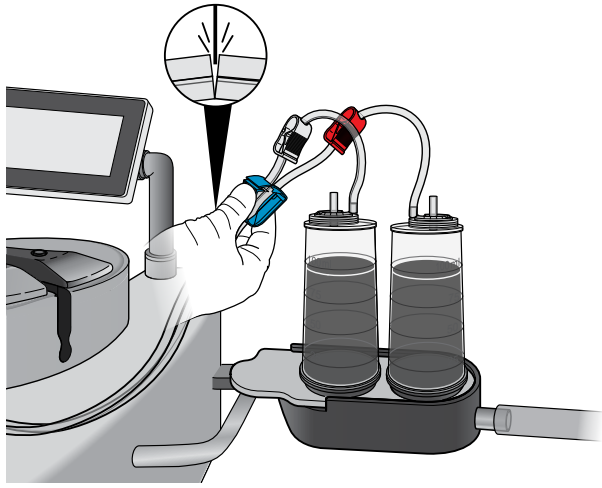
1. Ensure that all clamps are open.
2. Open the pump head.
3. Ensure that residual pressure is not flowing from the canisters (right side) to the empty medium bottle (left side). Drops of medium remaining on the tubing and suddenly moving from the right to the left indicate residual pressure in the canister.

   NOTE Residual pressure can be caused by a clogged canister vent.
4. Record the presence of residual pressure. If a positive result is achieved, residual pressure could be considered a deviation from the established procedure and be justification for retesting.
5. Close the white and red clamps located on the sides of the canisters to clamp off both tubing lines approximately 100 mm (3.9 in.) above the canisters.

   NOTE The white clamp identifies the tubing of the canister filled with TSB. The red clamp identifies the tubing of the canister filled with FTM. This color code reduces the risk of error during the incubation step: the red clamp is associated with the pink media, which is stored in a bottle with red cap.
6. Remove the tubing from the pump head, keeping the empty media bottle in the bottle holder.
7. Cut the tubing lines simultaneously by completely closing the Velax® blue cutting clamp approximately 30 mm (1.2 in.) from the closed clamps. This ensures that the canisters remain closed to the environment.

   NOTE Do not cut tubing where droplets of media remain. Slide the Velax® cutting clamp along the tubing to an area free of droplets.

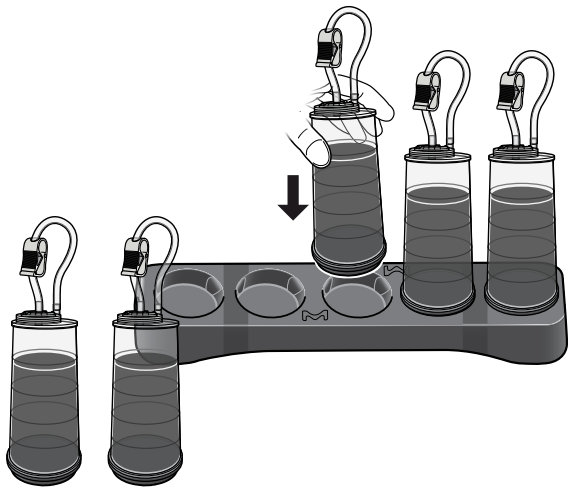
   Hold the Velax® cutting clamp, blade edge looking downward, between the thumb and the forefinger. Press vertically with the thumb to cut the tubings in one movement.



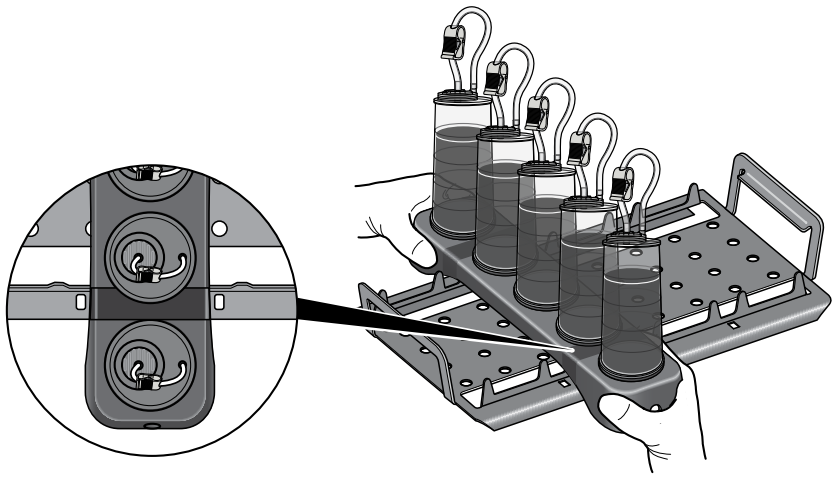
8. Fold the tubing over and insert the ends onto the vents of the canisters.

9. Remove the canisters from the drain tray and set them aside.

NOTE The Steritest® carrying tray (see Accessories for Sterility Testing) can be used to ensure the safe transport of the canisters from the test environment to the incubators.



NOTE The Steritest® rack (see Accessories for Sterility Testing) can be used to secure and transport up to four Steritest® carrying trays. Up to 20 media-filled canisters can be safely transported at one time.



10. Dispose of the Steritest® cutting clamp, tubing, needles, fluid bottles, sample containers, and filtrates in accordance with local regulations.

Reading the Canisters

1. Incubate the canisters for the time and temperature recommended by the relevant pharmacopeia.

   NOTES

   Incubation temperatures:
   20–25 °C for TSB
   30–35 °C for FTM

   Incubation duration: 14 days

   The Steritest® carrying tray and rack can remain in the incubator.

2. Observe for the presence or absence of turbidity to indicate the presence of microorganisms.

   NOTE During intermediate and final readings of the sterility test, gently swirl the canisters and search for the presence of floating bodies, which indicate the presence of microbial growth.

3. After incubation, dispose of the media-filled canisters in accordance with local regulations.

Troubleshooting

Problem	Possible cause	Remedy
Air bubbles are present in the tubing during liquid transfer.	Air is being aspirated into the sampling needle.	When using a separate vent needle, ensure that the vent needle is inserted deeper into the bottle septum than the sampling needle. This will prevent air aspiration into the sampling needle. Pre-wet the Steritest® device again and perform sample filtration a second time.
The liquid is not transfered or not evenly distributed between the two canisters.	The tubing is improperly positioned in the pump head.	Remove the bottle from the bottle holder and place it upright of the workbench. Stop the pump. Open the pump head and inspect the the tubing. If the tubing is not damaged, reinstall it in the pump head following the procedure described in Installing the Steritest® Device. If the tubing is damaged, replace the Steritest® device with a new one.
	Canister tubing is pinched or punctured.	
	The tubing is clamped. or One of the clamps is closed.	Remove the bottle from the bottle holder and place it upright of the workbench. Stop the pump. Ensure that none of the four clamps is closed.
	Pump speed is set too high.	Reduce the speed.
	The needle lumen is blocked by a fragment of septum.	Repeat the test with a new Steritest® device.

Liquid is not filtering through the canister membrane.	There is no pressure inside the canister. or The outlet port of the canister is plugged.	Ensure that the red caps are positioned properly on the vents of the canisters.
		Ensure that the yellow plugs are not installed on the bottom outlet ports of the canisters.
No air bubbles come out of the separate vent needle.	The separate vent needle does not go through the septum.	Push the separate needle fully through the septum.
	The separate vent needle is clogged by a piece of septum. or The separate vent needle filter is wet and no longer vents.	Replace the separate vent needle.
The tubing is pulled inside the pump head on the needle side.	The tubing is not placed correctly in the pump head.	Open the pump head and inspect the the tubing. If the tubing is not damaged, reinstall it in the pump head following the procedure described in Installing the Steritest® Device. If the tubing is damaged, replace the Steritest® device with a new one.
Red plugs keep popping off because of pressure inside the canisters.	The filtration membrane is clogged	Solubilize the canister content or optimize the test method.
	The yellow plugs are in place.	Remove the yellow plugs
	The pump speed is too high.	Reduce the pump speed.
During the rinsing step, the canister does not fill up, and the rinse fluid filters immediately.	The red caps are in place.	Remove the red caps.
	The canister vent is wet.	Discard the Steritest® unit and repeat the test.
Culture medium is leaking from the canisters during incubation.	The yellow plugs are not properly installed.	Ensure that the yellow plugs are installed properly. Twist them 90° while pushing them in place on the bottom outlet port of the canisters.
The FTM medium in the canister has a pink zone more than half the total height of the culture medium during the 14 days incubation	FTM culture medium has been introduced into the canister with a pump speed that is too high. Oxygenation has occurred during medium transfer.	Reduce the pump speed (30 maximum).
	The pump has remained on too long after the FTM has been transferred to the canister.	Stop the pump immediately after the FTM transfer.

Accessories for Sterility Testing

Description	Quantity Per Pack	Catalog Number
Steritest® Symbio Pump		
Steritest® Symbio LFH Pump Kit, 2 media	1	SYMBLFH01WW*
Steritest® Symbio ISL Pump Kit, 2 media	1	SYMBISL01WW*
Steritest® Symbio FLEX Pump Kit, 2 media	1	SYMBFLE01WW*
Steritest® Symbio Pump Accessories		
Steritest® pump footswitch	1	SYMBFSW01
Steritest® glass ampoule breaker	1	SYMBABR01
Steritest® pumps syringe support	1	SYMBSYS01
Steritest® Symbio waste overfill sensor for solid containers	1	SYMBWFS01
Steritest® carrying trays for 5 canisters	8	SYMBCAN08
Steritest® rack to hold up to 4 canister carrying trays	2	SYMBRACK2
Software (Available at http://www.merckmillipore.com/steritest-software)		
Steritest® Symbio Software	—	—
Consumables		
Steritest® culture media and rinsing fluids	Visit our website or contact our sales representative.	
Documentation (Available at http://www.merckmillipore.com/sterility-testing)		
Steritest® Symbio Pumps User Guide	1	PF16598
Steritest® Symbio Software User Guide	1	PF16600
Steritest® Symbio Pump Startup Quick Guide	1	PF16601
Steritest® Symbio Pump User Interface Quick Guide	1	PF16602
Steritest® Symbio Software Quick Guide	1	PF16603
Steritest® Syringe Support User Guide	1	PF17207
Steritest® Glass Ampoule Breaker User Guide	1	PF17206
Steritest® Syringe Support User Guide	1	PF17207
Steritest® Canisters Carrying Trays and their Rack User Quick Guide	1	PF17451
Interactive Steritest® brochure	1	MS_BR1495EN** MK_BR1495EN
Steritest® devices datasheet	1	MS_DS2971EN** MK_DS2971EN
Steritest® wall chart	1	MS_PS2737EN** MK_PS2737EN

* Country code to be defined at ordering step.

** Documents for US and Canada websites.

Standard Product Warranty

The applicable warranty for the products listed in this publication can be found at **www.sigmaaldrich.com/terms** (within the “Terms and Conditions of Sale” applicable to your purchase transaction).

Technical assistance

Please check the product webpage whether an update of this user guide is available.

For more information visit **www.sigmaaldrich.com/techservice**

Lit No. MS_UG11647EN Ver. 3.0
12/2022