

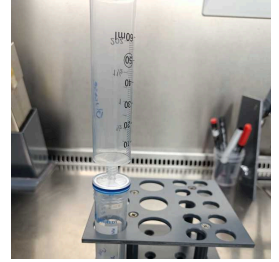


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Sterile filtration of liquid solutions

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Protocol status: Working

We use this protocol and it's working

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Abstract

Some liquids are not suitable for autoclaving but need to be sterile for use. Here we describe the process of filtering any solution to achieve sterility (at least removal of organisms bigger than 0.2 μm). In this protocol we show how we filter solutions in our lab that cannot be autoclaved, while we keep sterility, and reducing the amount of garbage.

Image Attribution

All images in this protocol are under Creative Commons License (CC 4.0 BY-SA-NC). They have been made by Christoph Meister, for the Exotoxins Lab.

Guidelines

1. As good microbiological practice, all containers used for components should be labeled with the name of the solution, the date of creation / aliquot, and initials of the person who did it, in order to ask questions if something is needed.
2. If you have doubts about how to perform a step in the protocol, consult your supervisor or someone who has prepared the solution before.
3. For any questions, feel free to email us (exotoxinslab-(at)- gmail.com). We are more than happy to help you.
4. Some of the protocols will be posted in our YouTube channel (www.youtube.com/@exotoxinslab).

Materials

- 50ml syringe x1
- 50ml Falcon tube x times
- Syringe filter x1 or x2
- Storage container (For example: 15ml Falcon tubes, Eppendorf microcentrifuge tubes)
-  PARAFILM® M Merck MilliporeSigma (Sigma-Aldrich) Catalog #P7793-1EA

Protocol materials

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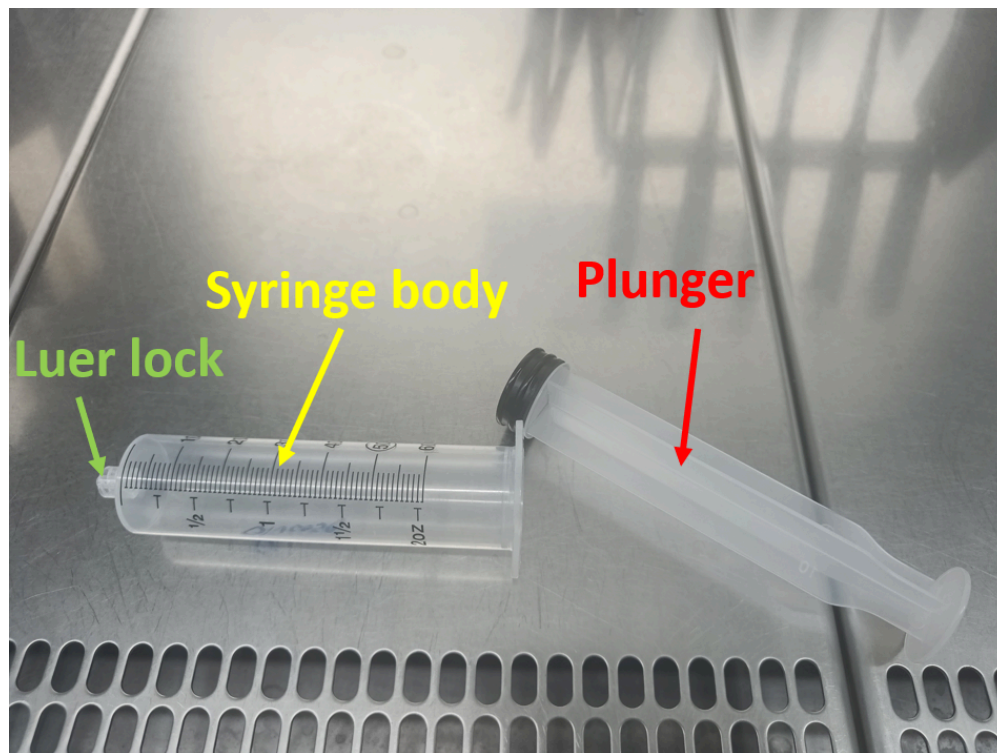


Before start

1. Be aware of your lab's safety protocols and check the attached Warnings (Guidelines and Warnings) to see what steps are necessary to protect yourself, such as protocol-appropriate personal protective gear, like lab coat, gloves and glasses.
2. Make sure to read the whole protocol before starting and have all the materials at hand.

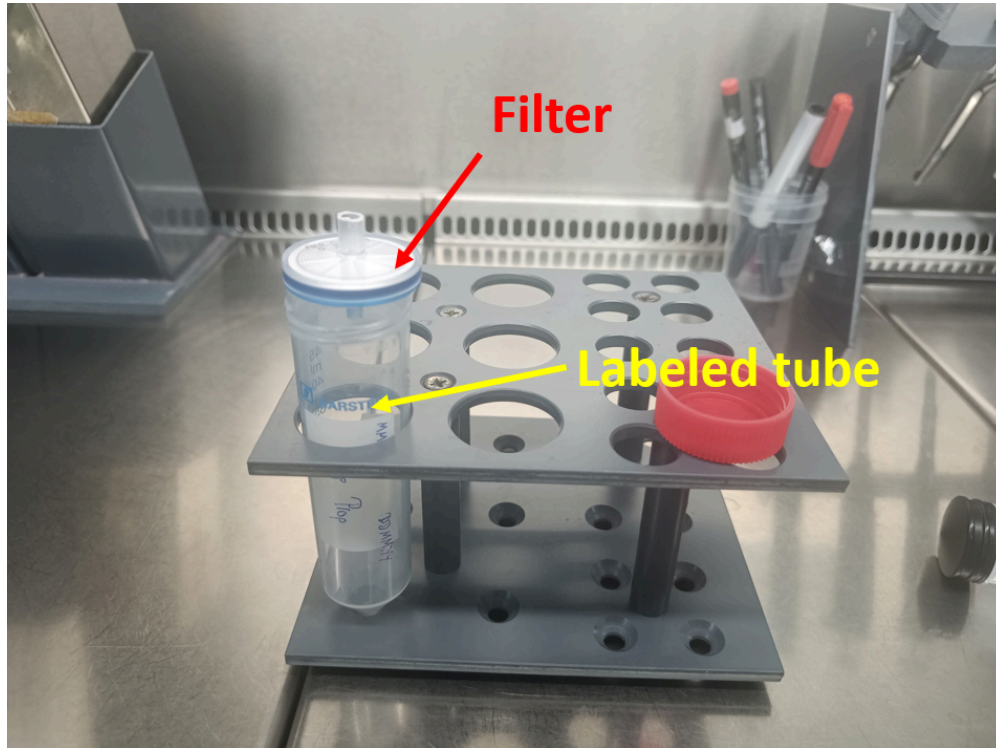
Filtration process

- 1 Turn on your biosafety cabinet and wait until laminar air flow has been established. Make sure you know how to work inside it so that sterility is guaranteed.
- 2 Put on gloves.
- 3 Place under the hood a syringe with luer lock, sterile conical tubes for liquid collection, 0.2 μm filter(s) and the solution you intend to filter .
- 4 Open the syringe package and **take the plunger out** of the syringe body.

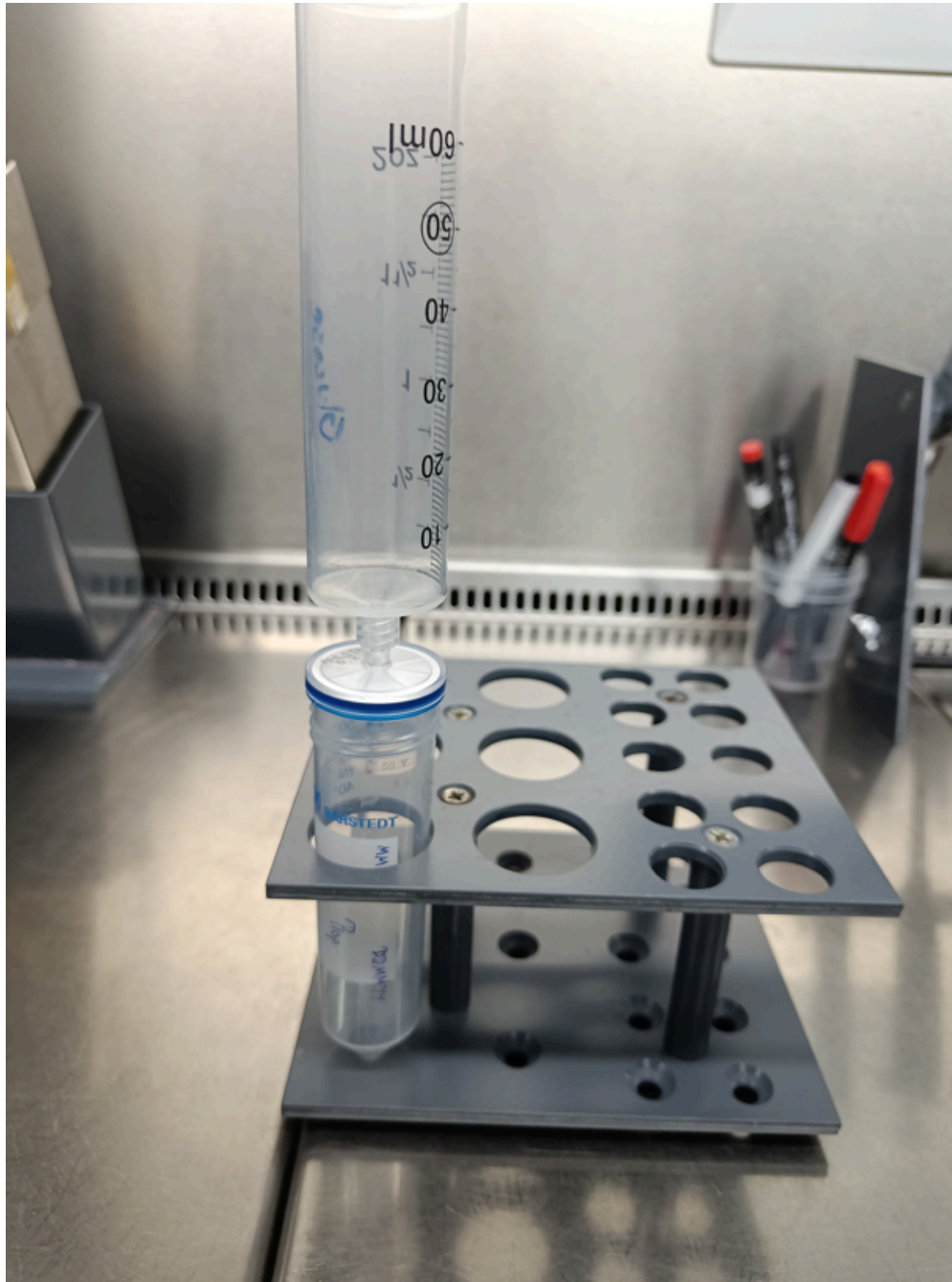


Parts of a 50 ml syringe with luer lock.

- 5 Open one 50 ml conical tube and place it in a rack. Make sure you do not touch the edge of the open tube.
- 6 Place a filter on the 50 ml tube you just opened.

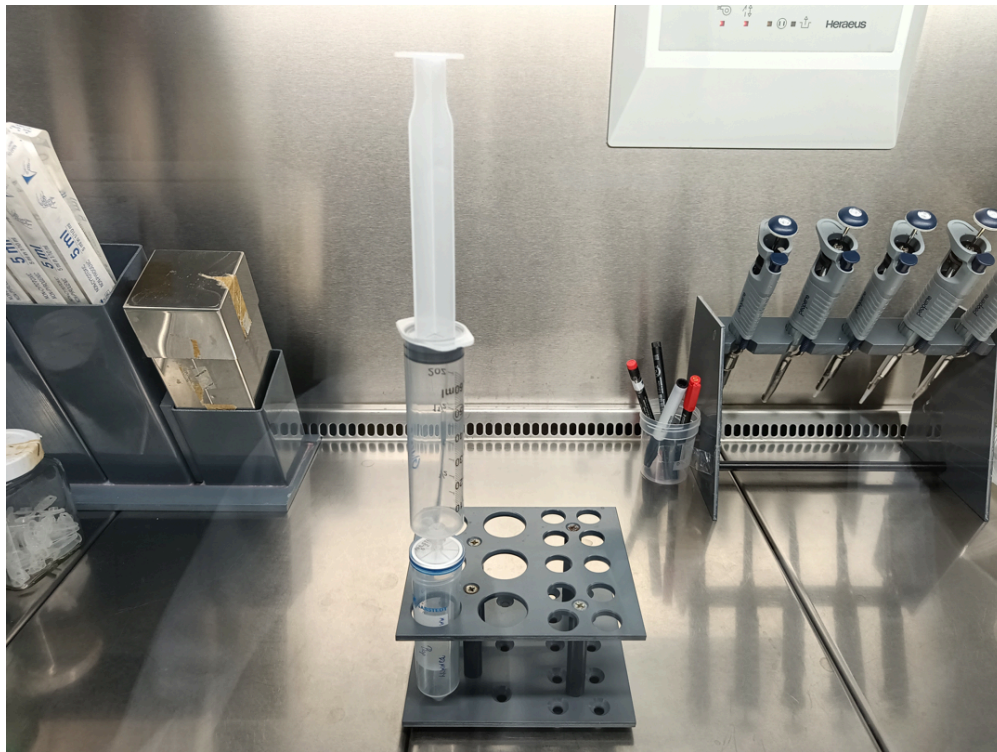


- 7 Connect the syringe body, which is **STILL WITHOUT THE PLUNGER** to the filter by screwing it on the luer lock. This connection guarantees a stable connection during filtration.



Luer lock from syringe screwed on to the filter, which was placed on top of the opening of a sterile conical tube.

- 8 Gently pour in 50 ml of the solution you want to filtrate inside the syringe.
- 9 Reconnect the plunger with the syringe body and push it down, so that the filtered liquid goes into the empty conical.



Set up shortly after inserting the plunger in the syringe containing the liquid to be sterile filtrated

Note

The insertion of the plunger requires a short use of force. Make sure you have stabilized the tube-filter-syringe construction properly before applying force or you might loose your solution or contaminate the tube.

- 9.1 Optional: In case you want to filtrate more then 50 ml.
- 9.2 Leave a small residue in the tip of the syringe. Do not press air into the filter.
- 9.3 **Unscrew** the syringe from the filter.
- 9.4 **Remove the plunger (only after unscrewing).**



- 9.5 **Screw** the syringe back on the filter.
- 9.6 Again pour in 50 ml of the rest of the solution you are filtering.
- 9.7 Reconnect the plunger and filter the liquid by pressing the plunger down. Make sure to not overflow your 50 ml conical tube. Use a second sterile tube if necessary.
- 10 When all liquid is filtered close the 50 ml conical.
- 11 If needed: Prepare your storage containers (15 ml conical tubes, eppis, etc.)
- 11.1 Aliquot the filtered solution into the storage container.
- 11.2 Close the storage containers with  Parafilm M **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P7793** . Parafilm allows us to make sure the liquid will not be spilled during storage, and a broke seal identifies the current aliquot in use.
- 12 **You can save the syringe for repeated use. Make sure to wash them properly with distilled water and let them dry. Label it with the name of the liquid you filtered, date and name abbreviation.**
- 13 Discard the other single use materials into the appropriate garbage. Store your filtered solution accordingly to recommended storage conditions.

Acknowledgements

We thank Dr. Walther Mothes for showing LFJS how to perform sterile filtration of liquid solutions while avoiding waste of resources.