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Chemical Decontamination and Maintenance of the BD FACS Aria Fusion Sheath Fluid Tank

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We use this protocol and it's working

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Abstract

Regular tank maintenance prevents formation of biofilms, rust, and contamination of cytometers or sorters.

This protocol has been tested as being as effective as autoclaving the tank, but without the minor risk of tank pressurization issues associated with applying high heat during the autoclaving process. It is ideal for core facilities that do not have access to an autoclave.

There are rare instances of contamination in which chemical-resistant biofilms can form on the interior of the metal sheath tubing within the tank. In these instances, autoclaving the tank is required. If autoclaving cannot remove the biofilm, then complete replacement of the tanks and parts would be necessary.

Image Attribution

Photos taken by Jamie Tijerina

Materials

2 Gallons/8L of Bleach
2 Gallons/8L of 200 proof ethanol
CaviWipes
Paper Towels
1000mL Graduated Cylinder
Adjustable Wrench
Teflon Tape
Lab Coat
Safety Goggles
Distilled White Vinegar
Stainless Steel Wire Brush
Mr. Clean Original Magic Eraser Cleaning Pads with Durafoam
Long Cotton Tipped applicators (Q-Tips)
Scissors
Razor Blade Scraper

Safety warnings

Wear a labcoat that is resistant to liquid. Wear gloves when handling the tank. Wear safety goggles to protect your eyes from potential splashing of bleach or ethanol. Ethanol must be rinsed off well or dried fully before applying bleach: mixing them can produce chloroform, which is toxic. Rinse bleach fully from stainless steel in order to prevent rust formation.

Before start

Ensure that you have access to a clean MilliQ or similar DI water dispenser. Do not use tap water. Ensure that you have access to a working biosafety cabinet. Ensure all consumable supplies listed in the materials section are stocked before starting the cleaning procedure.

1 Wipe down the surface of a biosafety cabinet with 70% Ethanol or CaviWipes. Switch on the air flow.

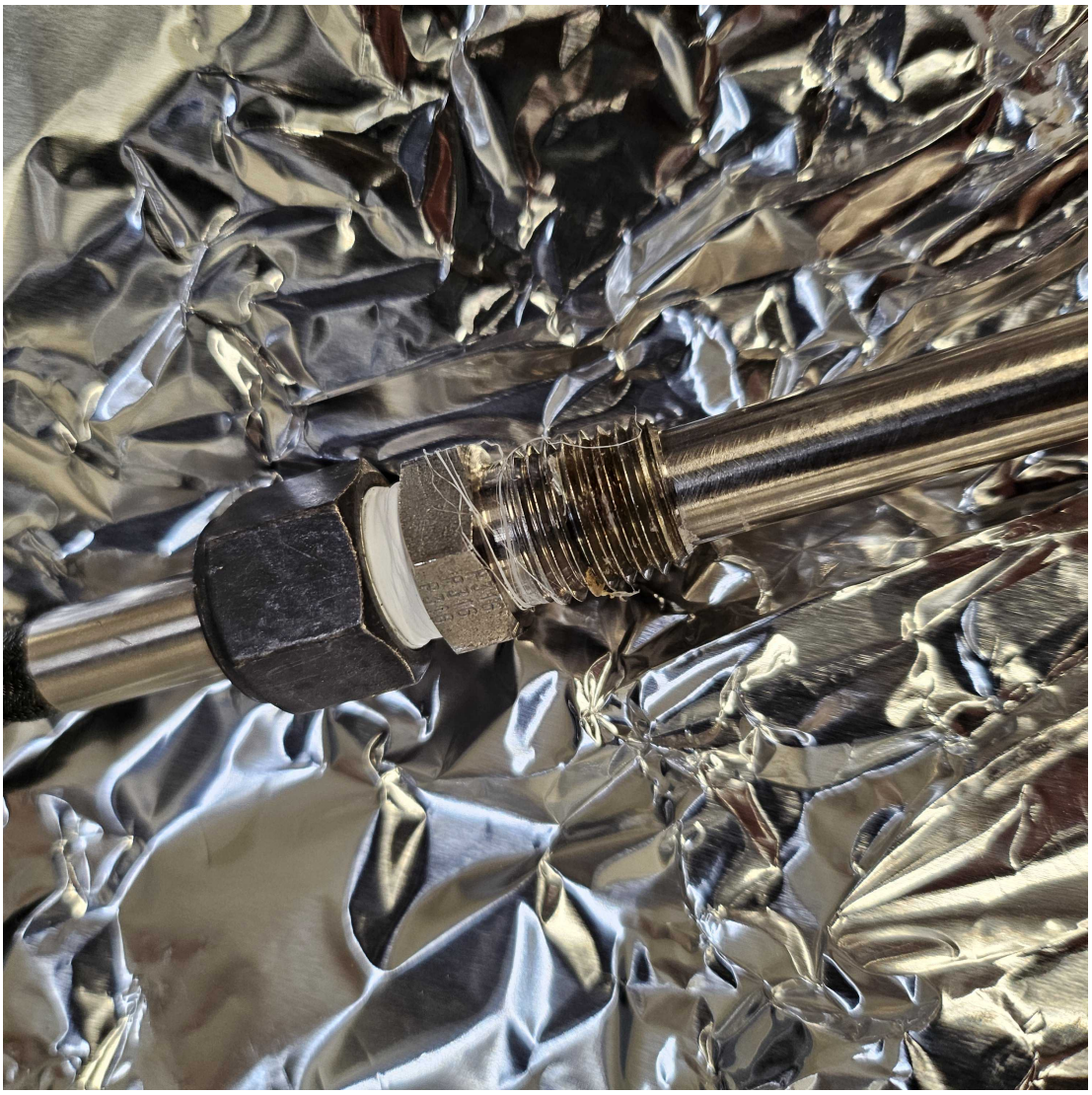
*Make sure that your biosafety cabinet is cleaned regularly, including underneath the grates and surface. You may need to lift these components out in order to do this. This should be done at least once a month.

*Perform as many steps as possible within the biosafety cabinet. It may be challenging to soak or rinse the tank inside the cabinet, but many other components can be placed to dry or soak there throughout the process.

2 Line the flat surface inside the biosafety cabinet with either CaviWipes or Paper Towels sprayed with 70% ethanol.

*Lining the surface with CaviWipes or ethanol-sprayed paper towels increases the contact time between the surface and the ethanol and/or disinfecting agents, as opposed to only wiping down the surface and placing the items to dry.

3 Carefully remove the sheath probe/sensor from stainless steel sheath tank using a wrench. Rest it on the lined surface. Remove all teflon tape residue, using q-tips and razor as needed to remove pieces.



This is a photo showing remnants of teflon tape on the thread. Make sure to remove all remnants.

4 Prepare 1000mL (1L) of 70% ethanol in a 1000mL graduated cylinder. Set aside 300mL in a separate container for other uses. Leave the cylinder filled with 700mL of 70% ethanol and place it inside the biosafety cabinet.

5 *This step is optional,* but useful if **contamination** is suspected, or if **rust** is observed: carefully remove the two connector fittings using a wrench. Remove all remaining pieces of teflon tape from the fittings.

If no rust is detected on the connectors: Soak them in 70% ethanol for 10 minutes in a small container using the 300mL of 70% ethanol prepared and set aside in the previous step. Once the 10 minutes has passed, rinse them with DI and place them to dry on a lined surface in the biosafety cabinet.

If rust is detected on the connectors: Soak the fittings in distilled white vinegar overnight in a small container. The next day, use a stainless steel brush to remove the rust easily from the connectors. Rinse with DI water and dry well.

If rust is detected on the surface of the tank: use Mr. Clean Magic Eraser to remove the rust. These are milder than steel wool and will remove most rust spots from the tank surface without damaging the tank finish.

6 Using a long Q-tip, razor, or similar instrument, remove any remaining pieces of teflon tape from the thread where the probe was seated and where the connector fittings were seated (if the connector fittings are removed).

7 Spray tank interior with 70% ethanol and allow to sit for 1-2 minutes.

*10 minutes is the ideal contact time for decontamination with 70% ethanol, but it is possible that the ethanol may dry out before 10 minutes. The main purpose of this step in this context is to remove any organic matter from the tank as this can inactivate bleach. 1-2 minutes has been sufficient for this purpose.

8 Rinse tank with filtered DI water twice. You do not have to fill the tank with DI, but you need to ensure that all ethanol and residue is removed to avoid any mixing of bleach and ethanol.

9 Rinse the sink basin to ensure any ethanol is cleared and down the drain to avoid mixing of bleach and ethanol.

10 Fill the tank all the way to the top with 12L of 25% bleach. Set a timer to 30 minutes. Allow the tank to soak for 30 minutes.

11 Remove the old paper towels from the biosafety cabinet surface. Then, once again line the surface in the biosafety cabinet with either CaviWipes or Paper Towels sprayed with 70% ethanol.

12 While the tank soaks, twist the plastic fittings off of the stainless steel lid. Wipe down the stainless steel lid, the plastic fittings, and the rubber o-ring with CaviWipes or 70% ethanol to remove any salt buildup or other dirt and organic matter.

*The o-ring may be replaced if it appears compromised and cannot be cleaned, or if it is more than 6 months old.

13 Place the stainless steel lid, the plastic fittings, and the rubber o-ring on top of the lined surface to dry.

14 Soak stainless steel portion of the lid in 2-4L of 25% bleach for 30 minutes. As long as ethanol has been allowed ample time to completely dry, then it is safe to place the lid in the bleach solution.

15 While the tank and lid components are soaking, wipe the tank exterior with CaviWipes or 70% ethanol.

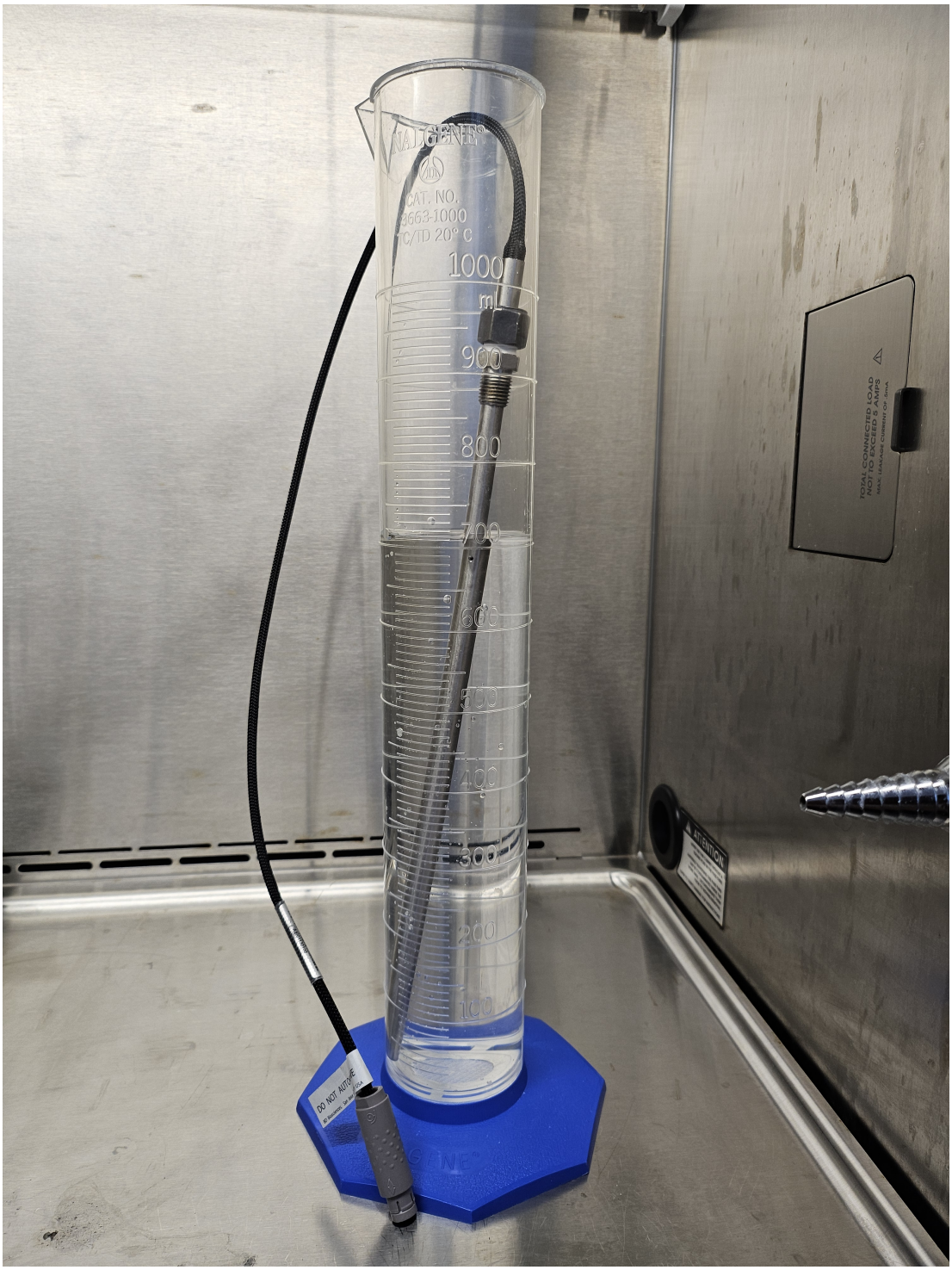
16 When the tank has reached 15 minutes into the soak, begin the process of cleaning the probe. Wipe down probe with CaviWipes or 70% ethanol.

*DO NOT use bleach on the probe - this is not recommended by BD and can damage it. The probe is costly to replace if damaged. Additionally, the sorter cannot operate if the sheath probe is not functioning and/or not installed properly in the tank.

*DO NOT pull the connector or the wire or expose it to liquid. Handle with care.

17 Take the probe to the graduated cylinder prepared with 700mL of 70% ethanol. Then place the probe in carefully in the cylinder, taking care to make sure that the connector and wire do not come in contact with the liquid.

18 Set a separate timer for 10 minutes. Allow the probe to soak for 10 minutes.



19 Remove the old paper towels from the biosafety cabinet surface. Then, once again line the surface in the biosafety cabinet with either CaviWipes or Paper Towels sprayed with 70% ethanol

20 After 10 minutes has passed, remove the probe from the cylinder and carefully rinse the probe with DI water. Then place it on the newly lined flat surface.

21 At this point, the timer for the soaking tank will likely be close to done with the 30 minute countdown. Once the timer countdown is complete, empty the 12L of bleach carefully into the sink. This tank will be heavy when filled completely. Then rinse the tank with filtered DI water twice. You do not have to fill the tank with DI, but you need to ensure that all bleach and residue is removed.

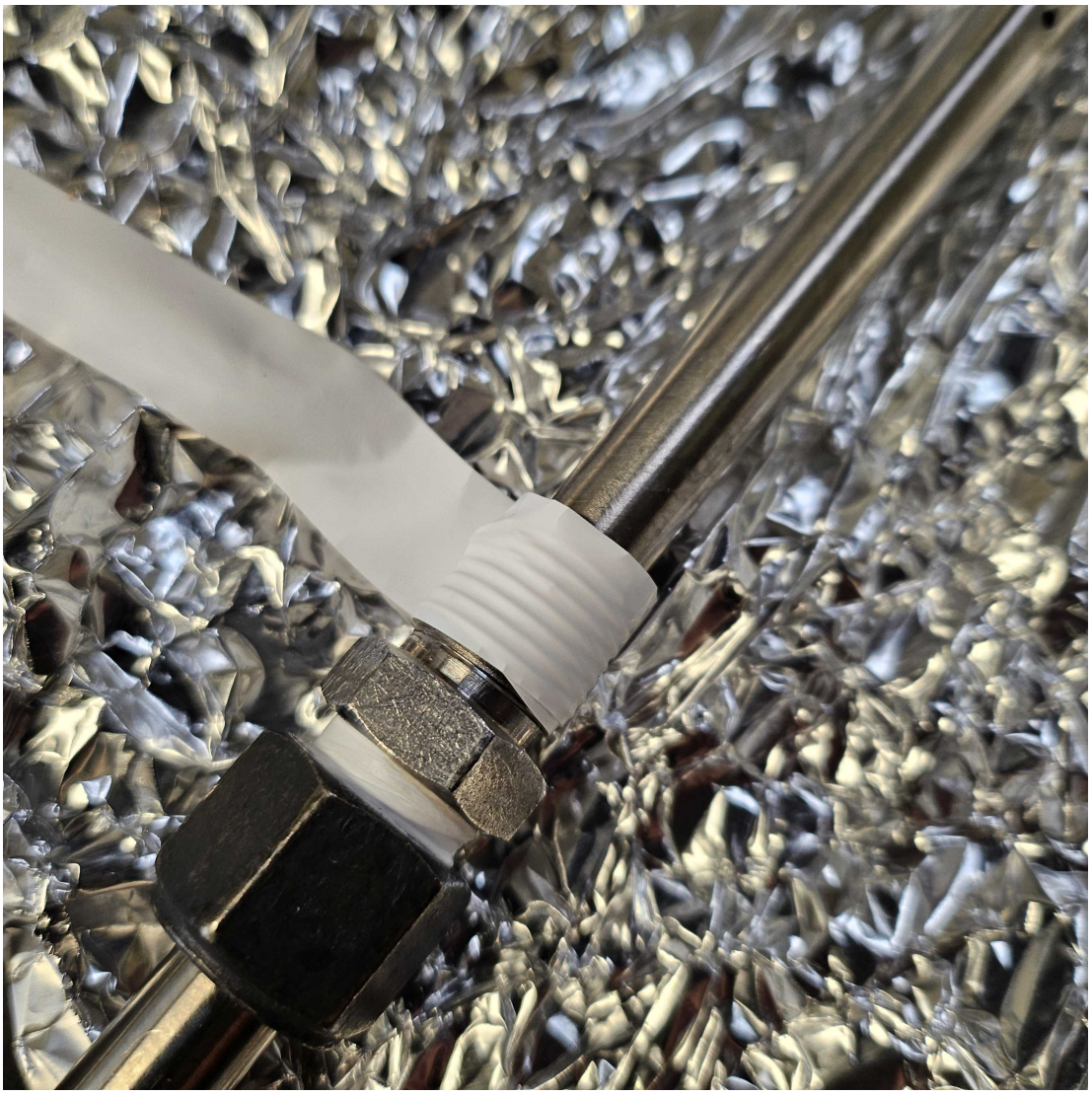
22 Rinse the sink basin to ensure any bleach is cleared and down the drain to avoid mixing of bleach and ethanol.

23 Once the tank is rinsed, re-insert the probe: ensure a proper seal by first wrapping the thread with teflon tape and tighten with wrench.

If the connections were also removed, wrap the thread for each with teflon tape and tighten with a wrench.

If the tank will be placed in storage, ensure that the tank is fully dried before re-placing the lid in order to prevent rust formation.

If the tank will be filled with sheath for use on the sorter, fill it immediately in a biosafety cabinet.



Correctly wrapped thread using fresh teflon tape.

24 At this point, the timer for the stainless steel lid will also have completed 30 minutes of soak time. Drain the container with the bleach and lid and then rinse the lid with DI water. The o-ring can be added back to the lid, and then the lid can then be placed on the tank. It is best to refill and close the tank inside a biosafety cabinet. Or, if the tank will not be immediately used and will be stored, it should be allowed to dry first in a biosafety cabinet.

Protocol references

BD FACS Aria III Users Guide
BD Application Note, Aseptic Sort Procedure
Note on Chloroform