Candida biofilm RNA isolation from venous catheters

1. Cut the end of the catheter into multiple 2- to 3- mm thick doughnuts with DEPC-treated scissors directly into buffer ?Are we still using AE?.
2. Place sliced catheter into eppendorf tube, and add 400 ul AE buffer.
3. Sonicate the tube for 10 minutes in a sonicating water bath, and vigorously vortex for 30 seconds.
4. Add 40 ul 10% SDS.
5. Add equal volume acid phenol (440 ul) warmed in heat block to 65˚C.
6. Incubate at 65˚C for 4-6 minutes, vortexing every 30 seconds.
7. Centrifuge at top speed for 2 minutes at room temperature.
8. Transfer aqueous phase to new 1.5 ml tube.
9. Repeat steps 5 through 8.
10. To aqueous phase, add equal volume phenol:chloroform:isoamyl alcohol (what volume?), vortex.
11. Centrifuge at top speed for 5 minutes.
12. Transfer aqueous phase to new tube, and add 1/10 volume 3 M NaOAc, pH 5.2, and 2.5 volumes 100% EtOH (-20˚C) (what volumes?).
13. Store at -20˚C for 15 minutes to precipitate.
14. Centrifuge at top speed for 5 minutes, carefully remove supernatant.
15. Resuspend pellet in 400 ul DEPC-treated H2O.
16. Add 40 ul 3 M NaOAc, pH 5.2, 1 ml 100% EtOH (-20˚C), and place at -20˚C for 15 minutes.
17. Centrifuge at top speed for 5 minutes, carefully remove supernatant.
18. Wash pellet with 500 ul 80% EtOH, place at -20˚C for 15 minutes.
19. Centrifuge at full speed for 3 minutes, carefully remove as much supernatant as possible.
20. Air dry pellet, 10-15 minutes or as needed.
21. Resuspend in 20 ul H20 (DEPC-treated?).
22. Store at -80˚C.

AE Buffer:
50 mM NaOAc, pH 5.3

10 mM EDTA

Acid Phenol: Ambion Cat. No. 9720
Phenol:CHCl3:IAA: Ambion Cat. No. 9730 (do NOT raise pH with provided buffer)