Modular On-Demand Water Purification for Developing Countries

2021-2022 Challenge Water Makeup Procedures

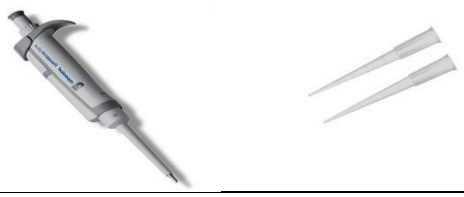
Challenge Water Makeup

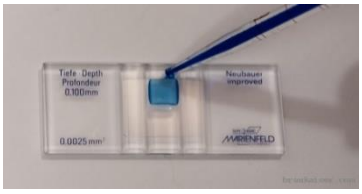
The challenge water will have the characteristics/components listed in table 1, shown below:

Table 1: Challenge Water Characteristics/Components

Component	Amount/Value	Purpose	Notes
Brewer's Yeast	$\geq 10^7$ per 100 mL	Surrogate for bacteria	Champagne yeast recommended. Deactivated with heat prior to competition to stabilize concentration.
—	$\geq 10^7$ per L	Theoretical virus loading	Virus not included in challenge water, but must assume it is present
Polymer microspheres	$\geq 5 \times 10^4$ spheres per L	Surrogate for oocysts	6 µm polystyrene microspheres; from NSF P248 w/ modification
Chlorine	≤ 0.1 mg/L	Background chlorine	Adjustment should not be necessary if using DI/distilled water
NaOH/HCl	Adjust for pH of 7.0 ± 0.5	Adjust pH	Adjustment should not be necessary if using DI/distilled water
Tannic Acid	10-15 mg/L	Adjust TOC	NSF P248
Test Dust	Adjust to 50-100 NTU	Adjust Turbidity	Use ISO 12103-1, A2 fine test dust
Temperature	20 ± 5°C	Simulate typical ambient	Effectiveness of some disinfection methods depend on temperature
NaCl	1500 ± 300 mg/L	Adjust TDS	NSF P248

Required Equipment

Micropipettes (0.5 µL- 10 µL) and pipette tips	

Beakers	
Graduate Cylinders	
Light Microscope	
Hemocytometer	
Eppendorf tube	
Hotplate & Magnetic Stirrer	

Turbidity meter kit	
Digital Balance	

Required Materials

1. Champagne yeast (Saccharomyces cerevisiae)
2. Dyed Microsphere 6.00µm (refrigerate until use)
3. Kosher Salt
4. A2 Fine Test Dust
5. Tannic Acid, 95%
6. Brewing Sanitizer
7. Methylene Blue Stain 1% solution
8. Distilled or DI water

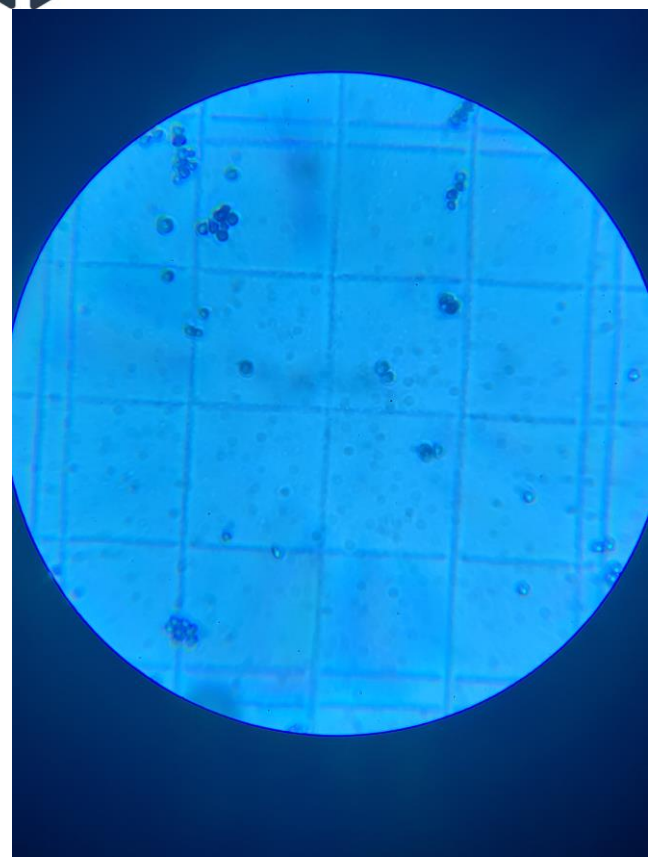
Challenge water makeup excel sheet

Challenge water Makeup.xlsx

Procedures

1. Sanitize all equipment that will come in contact with the yeast or final challenge water with dilute brewing sanitizer. **Follow dilution and washing directions on the brewing sanitizer packaging.**
2. Dissolve a packet of champagne yeast in 50 mL of water at 35-40 °C, as directed on the packet of brewing yeast.
3. Once the yeast is dissolved, bring the temperature of the solution up to 90-95 °C using the hot plate and hold for 15 min to deactivate the yeast. Do the following once the yeast has been deactivated:
    a. Dilute the yeast solution by a dilution factor of 100 starting with an empty Eppendorf tube and add 10 µL of the well-mixed yeast solution, 940 µL of Distilled or DI water and one drop (50 µL) of 1% methylene blue solution in to the Eppendorf tube.
    b. Adjust the microscope's slide clamps upward to accept the hemocytometer.
    c. Clamp in the hemocytometer with the slide cover.
    d. Add 10 µL (or enough to ensure the counting plate is completely covered) of the diluted & stained yeast solution to the hemocytometer.
    e. Count the cells, record the cell count, calculate cell concentration, and assess the viability using the method listed here and Challenge water Makeup.xlsx, attached above. Use an average of multiple counting squares.
    Note: count cell clusters as 1 colony forming unit (cfu)

    Below is an image showing it may look like:



f. Repeat steps 3.a-e for 3 separate vials and on both sides of the hemocytometer to reduce error in measurement. To reduce risk of contamination, wash the hemocytometer with isopropyl alcohol, denatured ethanol, or brewing soap between measurements. Take the average of the three cell counts for step 4.

4. Based on the concentration measured in step 3.e, calculate the amount of the yeast solution that would be needed to achieve a concentration of 10^8 cells/L, or 10^5 cells/mL using the formula below. The count volume is 0.004 µL due to the dimensions of the hemocytometer squares being 0.2 mm x 0.2 mm x 0.1 mm.

$$Concentration = C = \frac{average\ count}{count\ volume} * Dilution\ factor = \frac{average\ count}{0.004\ \mu L} * 100$$

Note: This formula gives concentration as cells/µL. Multiply by 1,000 to convert to cells/mL. An excel sheet that calculates cell counts and dilutions is attached.

5. Calculate the amount of microsphere solution needed to reach the concentration of 5 x 10^4 spheres per L. Successive dilutions are detailed in the excel document.

6. Begin preparing the challenge water by adding the tannic acid and salt to a 1-L vessel
   Note: Due to the inaccuracy of the scale at lower weights, a tannic acid solution must be made up and diluted as part of the 1 L challenge water solution. Perform steps 6.a & 6.b

   a. Completely dissolve 50 mg of tannic acid to 100 mL of water.
   b. Add 20-30 mL (depending on the desired final concentration) of that solution to the challenge water vessel.
7. Add approximately 500 mL of distilled or DI water to the mixture.
8. Add the microsphere and yeast solutions in amounts calculated in steps 4 and 5.
9. Bring the total volume of the solution up to 1 L.
10. Measure the turbidity of the solution.
11. Adjust the turbidity with test dust until it is within the range of 50-100 NTU, depending on the desired final turbidity.
    Note: ensure that the solution is constantly mixed before giving the solution to competitors. Discard the remaining 50-100 mL of solution if you are using magnetic stirring. This is due to the accumulation of ferrous metals from the test dust being pull out of suspension by the magnet and accumulating on the bottom of the vessel.

Below is an image of the test water before (right, ~6 NTU) and after (left, ~ 78 NTU) adding test dust.