SERIAL DILUTIONS – TUBE METHOD

Principle

Serial dilution is a common technique used in many immunologic procedures. A small amount of serum or solute can be serially diluted by transferring aliquots to diluent. One of the most common series doubles the dilution factor with each transfer (1:2, 1:4, 1:8 ...). These dilutions can be done in microtiter plates or test tubes depending on the volumes of sample and diluent used.

Materials

6 plastic test tubes
1 test tube containing 2 mL blue dye (already prepared)
1 test tube of distilled water

Procedure

1. Assemble the above materials at your workbench.
2. Label tubes for serial dilutions as follows:
   #1 (1:2); #2 (1:4); #3 (1:8); #4 (1:16); #5 (1:32); #6 (1:64)
3. Using a micropipettor, pipet 1 mL of distilled water into tubes #1, #2, #3, #4, #5, #6.
4. Be sure cap is firmly closed and mix the dye solution by inverting the tube. Using a micropipettor, pipet 1 mL of blue dye into tube #1. Mix gently by drawing the solution up and down 3 times (3X).
5. Transfer 1 mL of solution from tube #1 into tube #2. Mix **gently** 3X.
6. Transfer 1 mL of solution from tube #2 into tube #3. Mix **gently** 3X. Continue to transfer and mix through tube #6.
7. Discard the last 1 mL from tube #6.
8. Examine the tube dilutions. Note that the color decreases with increasing tube number.

Interpretation

Observe the color dilution progression. Assuming the dye was diluted 1:2 before use, record the correct serial dilutions for each tube.

Formula

Dilution = sample volume:total volume = sample volume: sample volume + diluent volume

example 1:2 dilution = 1 part sample:2 parts total

Diluent Volume = Total Volume - Sample Volume
Diluent Volume = 2 - 1
Diluent Volume = 1