Answers to Simple Dilution Problems

1) a. 1/10  
b. 1/100  
c. 1/4  
d. 1/2.5 or 2/5

2) a. 100 times 
b. 10 times  
c. 20 times  
d. 11.76 times

3) a. 1 mg/ml 
b. 0.2 mg/ml  
c. 16.83 mg/ml

4) 0.1 ml into 9.9 ml 3 times.

Answers To Advanced Dilution Problems

1) a. 75 plaques/ml of $10^{-3}$ --> $75 \times 10^3$ --> $7.5 \times 10^4$ pfu/ml  
b. 41/0.1 ml --> 410/ml of $10^{-6}$ --> $410 \times 10^6$ --> $4.1 \times 10^8$ pfu/ml  
c. 30/100 µl --> 30/0.1 ml --> 300/ml of $10^{-4}$ --> $300 \times 10^4$ --> $3.0 \times 10^6$ pfu/ml  
d. 26/50 µl --> 52/0.1 ml --> 520/ml of $10^{-8}$ --> $520 \times 10^8$ --> $5.2 \times 10^{10}$ pfu/ml  
e. 30/ml --> 30 x 5 --> 150 pfu/ml

2) 2. Take 0.1 ml from stock and mix with 9.9 ml of broth to get a $10^{-2}$ dilution. Take 0.1 of this and mix with 9.9 of new broth to get $10^{-4}$ dilution. Take 0.1 ml of $10^{-4}$ dilution and mix with 9.9 ml of broth to obtain a $10^{-6}$ dilution. Take 1.0 ml of $10^{-6}$ dilution and mix with 9.0 ml of broth to get $10^{-7}$ dilution. This last dilution produces 20 cells per 50 µl; therefore it contains 40 cells/0.1 ml or 400 cells/ml. Thus the original solution has $400 \times 10^7$ --> $4.0 \times 10^9$ cells/ml.

3) Can use the formula $CV = C'V'$
   $500 \text{ ml} \times 1.5 \times 10^2 \text{ cells} = 2.0 \times 10^6 \text{ V'}$
   $V' = (7.5 \times 10^4)/(2.0 \times 10^6) = 0.0375 \text{ ml} = 37.5 \mu l$

4) Taking 0.1 ml and seeing 36 colonies at $10^{-6}$ dilution means we have 360 cells/ ml at this dilution. So in the original solution there were 360 $\times 10^6$ bacteria/ml --> $3.6 \times 10^8$. We had 10 ml of the original solution, so there were a total of $3.6 \times 10^9$ bacteria present. Since 1000 bacteria weigh 5 ng, thus the weight of these bacteria is $(3.6 \times 10^9 \times 5)/1000 = 1.8 \times 10^7$ ng --> $1.8 \times 10^4$ µg --> $1.8 \times 10^1$ mg = 18 mg

5) Titer of the original solution is obtained from # plaques at time 0' (or 1' which is the closest). Thus 20/0.1 ml --> 200/ml of $10^{-4}$ --> $200 \times 10^4$ --> $2.0 \times 10^6$ pfu/ml. The relative titers and burst size are as follows:
<table>
<thead>
<tr>
<th>Time</th>
<th>Dilution Factor</th>
<th>#plaques</th>
<th>Relative titers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 min</td>
<td>$10^{-4}$</td>
<td>$2.0 \times 10^6$</td>
<td>2.2 $\times 10^6$=1.00</td>
</tr>
<tr>
<td>5 min</td>
<td>$10^{-4}$</td>
<td>$2.1 \times 10^6$</td>
<td>2.2 $\times 10^6$=1.00</td>
</tr>
<tr>
<td>10 min</td>
<td>$10^{-4}$</td>
<td>$2.5 \times 10^6$</td>
<td>2.2 $\times 10^6$=1.00</td>
</tr>
<tr>
<td>20 min</td>
<td>$10^{-4}$</td>
<td>$4.5 \times 10^6$</td>
<td>2.05</td>
</tr>
<tr>
<td>30 min</td>
<td>$10^{-4}$</td>
<td>$6.5 \times 10^6$</td>
<td>2.95</td>
</tr>
<tr>
<td>40 min</td>
<td>$10^{-5}$</td>
<td>$8.0 \times 10^6$</td>
<td>3.64</td>
</tr>
<tr>
<td>50 min</td>
<td>$10^{-5}$</td>
<td>$1.2 \times 10^7$</td>
<td>5.45</td>
</tr>
<tr>
<td>60 min</td>
<td>$10^{-5}$</td>
<td>$2.0 \times 10^7$</td>
<td>9.09</td>
</tr>
<tr>
<td>70 min</td>
<td>$10^{-5}$</td>
<td>$2.2 \times 10^7$</td>
<td>10.00&lt;&lt; burst size</td>
</tr>
<tr>
<td>80 min</td>
<td>$10^{-5}$</td>
<td>$2.1 \times 10^7$</td>
<td>9.55</td>
</tr>
</tbody>
</table>

6) Since we have 1g Amp/10 ml, our stock is 100 mg/ml and also since we need only 50 mg for the whole liter, we take 0.5 ml of our stock and add it to the autoclaved medium. We also can do this according to the formula $C_1 \times V_1=C_2 \times V_2$:

Take stock as 1 and diluted preparation as 2. Here are what we have:
- $C_1= 1$ g/10 ml= 0.1 g/ml= 100 mg/ml
- $V_1= We do not have this and want to calculate it
- $C_2= 50$ mg/1 L= 50 mg/1000 ml=0.05 mg/ml
- $V_2= 1$ L= 1000 ml

Thus $V_1= (C_2 \times V_2)/C_1 = (0.05 \times 1000)/100 = 0.5$ ml

7) The 0.5 O.D. corresponds to 1500 cells/ml. Since this came from a $10^{-3}$ dilution, the conc. of cells in original solution is $1500 \times 10^3 = 1.5 \times 10^6$ cells/ml.

8) (a) The fastest way is to find the dilution factors and multiply the number of cfu by the reciprocal of the dilution factors. The dilution factors are: 2/5, 10/1000 and 50/1000. The last one is not actually a dilution factor but is a conversion factor because we take 50 microliter but want the answer in ml. So $82 \times 1000/50 \times 1000/10 \times 5/2 = 82 \times 2.5 \times 100 \times 20 = 4.1 \times 10^5.$
   (b) No. That number is too low to cause any visible turbidity. We need to have at least $10^7$ bacteria to see the start of turbidity.

9) 0.1 ml soln and 9.9 ml of diluent $\Rightarrow 10^{-2}$
   0.1 ml new soln and 9.9 ml of diluent $\Rightarrow 10^{-4}$
   0.1 ml of $10^{-4}$ soln and 9.9 ml of diluent $\Rightarrow 10^{-6}$
   1.0 ml of $10^{-6}$ soln and 9 ml of diluent $\Rightarrow 10^{-7}$

10) 25 mg/ml; 125 mg/500 ml $\Rightarrow 0.25$ mg/ml = 250 micrograms/ml.

11) $10^{-4}$

12) (a) This is a trial and error method.
    First trial: Add 1 microliter of the sample to 999 microliters of diluent to make 1 ml of a $10^{-3}$ dilution, then add 1 microliter of $10^{-3}$ dilution to 999 microliters of diluent to make 1 ml of a $10^{-6}$
dilution. Finally add 0.1 ml of the $10^{-6}$ dilution to 0.9 ml of diluent to make 1 ml of $10^{-7}$ dilution. We have used just 1 microliter of the sample and 2898 microliters of diluent. This is too much diluent. Let’s see if we can decrease it.

(b) Second trial: Add 1 microliter of the sample to 99 microliter of diluent to make 100 microliter of a $10^{-2}$ dilution, then add 1 microliter of $10^{-2}$ dilution to 99 microliter of diluent to make 100 microliter of a $10^{-4}$ dilution. Then add 1 microliter of $10^{-4}$ dilution to 99 microliter of diluent to make 100 microliter of a $10^{-6}$ dilution. Finally add 10 microliter of the $10^{-6}$ dilution to 90 microliter of diluent to make 0.1 ml of $10^{-7}$ dilution. We have used a total of just 387 microliters of diluent and again only 1 microliter of the sample.

Note that the second trial is much better than the first. Try to see if you can find an even better way.

13) We should theoretically repeat the count with a less concentrated sample because we know that we should not use count data below 30 and above 300. However, if we have by mistake thrown away the original sample we were sent and it is hard to get a new sample, we could estimate the number of colony forming units to be 4500/ml in the lake.

14) If you find a certain number of cells in a sample and dilute the sample 100 times, we have to use 100 times as much of the diluted sample to see the same number of cells. This can also be solved by other ways; e.g. $5 \times 1 = Y \times 1/100$; $Y = 500$ ml.

15) $1/5 \times 1/10 \times 1/20 = 1/1000$.

16) Use the formula $C\ V=C'\ V'$. Thus $50\ \mu l \times 2.5\ mM = Y \times 20\ mM$. $Y = 50 \times 2.5/20 = 6.25\ \mu l$. This means we should take 6.25 $\mu l$ of each base stock and add 43.75 $\mu l$ of water.

17) One way to do this is to take 10 $\mu l$ of 20 mg/ml stock and bring up to 1 ml with water in one of the Eppendorfs to get a conc. of 200 $\mu g/ml$. Take 10 $\mu l$ of the new solution into the second Eppendorf and bring it up to 1 ml. This would give you a 2 $\mu g/ml$ solution.

18) 10 L weighs 2 $\mu g$. Every million cells weigh 8 $\mu g$, so 2 $\mu g$ of cells is 1/4 of a million, or 250,000 (or 2 x 1,000,000/8). Now 250,000 cells are from 10 L, so 1 ml should have 250,000/10,000 = 25 cells/ml.

19) (a) Conc of tet in well 1= 1.5 $mg/ml$
Conc of tet in well 2= 375 $\mu g/ml$
Conc of tet in well 3= 93.75 $\mu g/ml$
Conc of tet in well 4= 0.0

(b) $MIC = 375\ \mu g/ml$ of tetracycline