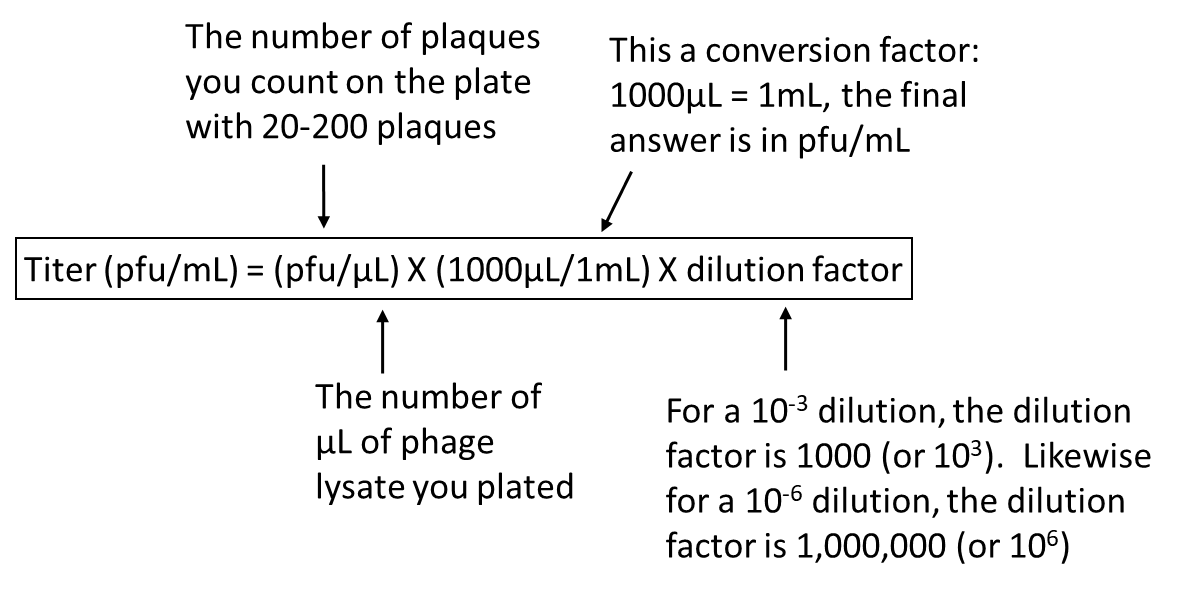
Phage Titer Calculations

In the Phage Discovery lab, we will often need to determine the number of plaque forming units per mL of phage lysate (pfu/mL).  This gives an indication of the number of ‘infectious’ phage particles in a particular sample and is a useful number for setting up subsequent infection experiments and when generating a high titer stock for isolating DNA.

To calculate a phage titer, you will need three experimental pieces of information.

1. The volume of sample added to the bacterial culture and subsequently plated on an agar plate.  (For our experiments this number will most likely be 10 µL.)
2. A count of the number of plaques on the plate.  A plate is considered suitable for counting if it has a range of 20-200 plaques.
3. The dilution of the counted plate.

Then use the following equation:



**Worked example:**  Darrell prepared 5 microcentrifuge tubes.  The first was his neat phage Owens lysate (100).  He then performed 10-fold serial dilutions in the remaining tubes and used 10 µL of the appropriate dilution to infect *M. foliorum*.  At 48 hours later, he checked his plates and counted colonies for 3 of the plates.  The following was recorded in his laboratory notebook.  Calculate the titer (pfu/mL) for each plate counted.

**Solution:**

| Phage Owens     Plate Dilution | Sample Vol | Number of pfu | Dilution Factor | Titer (pfu/mL) |
| --- | --- | --- | --- | --- |
| -1 | 10 µL | Too many to count | 10 | Cannot be calculated |
| -2 | 10 µL | 198 | 100 | (198 pfu/10 µL) X (1000 µL/1 mL) X 100 = 1.98 x 106 pfu/mL |
| -3 | 10 µL | 29 | 1,000 | (29 pfu/10 µL) X (1000 µL/1 mL) X 1,000 = 2.9 x 106 pfu/mL |
| -4 | 10 µL | 4 | 10,000 | (4 pfu/10 µL) X (1000 µL/1 mL) X 10,000 = 4.0 x 106 pfu/mL |

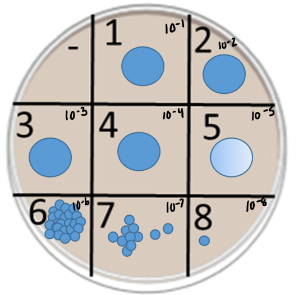
Practice questions

1. Did you notice that the titer calculated in the example shown on the chart on the previous page is similar but not exactly the same for each plate? Explain why that might be the case?

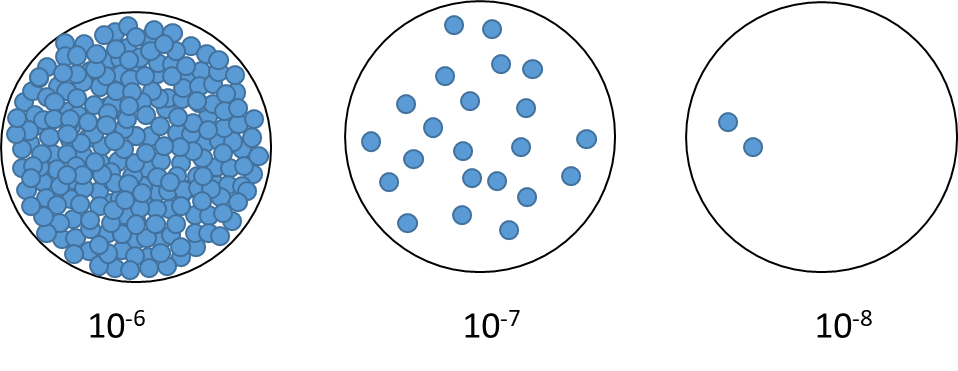
2. Practice Calculating Phage Titers

Calculate the titer (pfu/mL) for each line of data in this student’s notebook.

| **Phage Name** | **Sample Vol** | **Dilution** | **Number of pfu** | **Dilution Factor** | **Titer Calculation** |
| --- | --- | --- | --- | --- | --- |
| Snoopy | 10 µL | 10-3 | 45 |  |  |
| RollTide | 10 µL | 10-2 | 168 |  |  |
| Hammy | 5 µL | 10-3 | 39 |  |  |
| WarEagle | 10 µL | 10-6 | 132 |  |  |
| DeBarni | 5 µL | 10-5 | 101 |  |  |

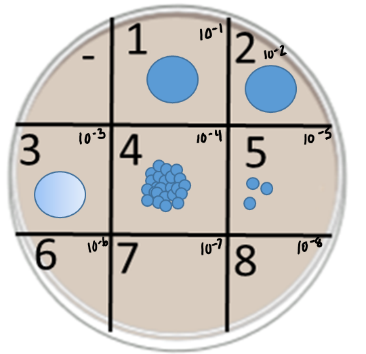
3.  Anita did 8 1:10 dilutions of her high titer lysate (HTL) and spotted 5µL of each dilution on a plate with a lawn of host bacteria.    


Next, Anita did a full plate titer assay for the 10-6 through 10-8 dilutions. She plated 10 µL of diluted lysate on each plate. Below are the results of the serial dilutions that Anita plated.



What is the titer of her HTL?  Show your work and include the units.

5. Based on the information from the full plate titer assay, how many plaques do you estimate are present on the 10-6 spot on the titer assay spot test?

6.  Anita needed more phage lysate to isolate phage DNA. She flooded the 10-6 plate to make a new lysate and then isolated phage DNA from the new lysate and then did a spot titer experiment using the new lysate. A DNA isolation experiment works best if the HTL has a concentration of at least 109 pfu/mL.  Anita’s spot test is shown below (she used the same protocol as described in question 3).  Is her lysate concentrated enough for DNA isolation?  Explain your answer.   
  


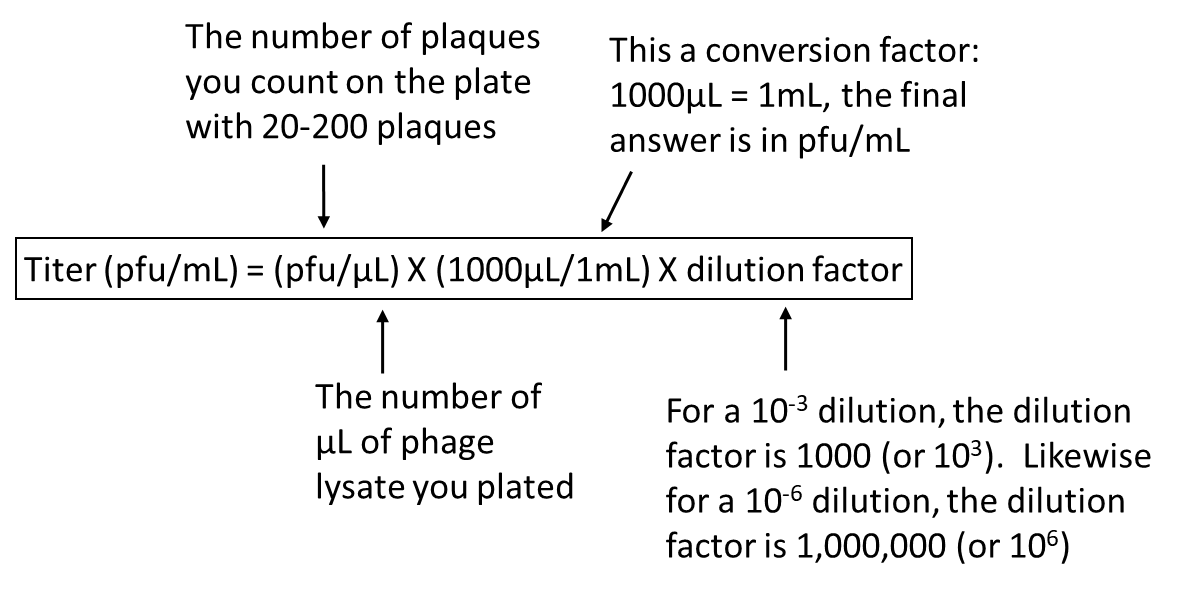
ANSWER KEY Phage Titer Calculations

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To calculate a phage titer, you will need three experimental pieces of information.

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Then use the following equation:



**Worked example:**  Darrell prepared 5 microcentrifuge tubes.  The first was his neat phage Owens lysate (100).  He then performed 10-fold serial dilutions in the remaining tubes and used 10 µL of the appropriate dilution to infect *M. foliorum*.  At 48 hours later, he checked his plates and counted colonies for 3 of the plates.  The following was recorded in his laboratory notebook.  Calculate the titer (pfu/mL) for each plate counted.

**Solution:**

| Phage Owens     Plate Dilution | Sample Vol | Number of pfu | Dilution Factor | Titer (pfu/mL) |
| --- | --- | --- | --- | --- |
| 10-1 | 10 µL | Too many to count | 10 | Cannot be calculated |
| 10-2 | 10 µL | 198 | 100 | (198 pfu/10 µL) X (1000 µL/1 mL) X 100 = 1.98 x 106 pfu/mL |
| 10-3 | 10 µL | 29 | 1,000 | (29 pfu/10 µL) X (1000 µL/1 mL) X 1,000 = 2.9 x 106 pfu/mL |
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Practice questions

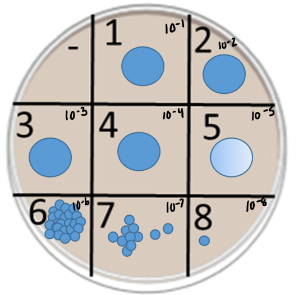
1. Did you notice that the titer calculated in the example shown on the chart on the previous page is similar but not exactly the same for each plate? Explain why that might be the case?

The same phage lysate (with the same titer) was used on all the plates so the magnitude of the titer calculated should be the same. Any discrepancies could be due to experimental error for example slight inaccuracies in pipetting or difficulty counting a large number of plaques.

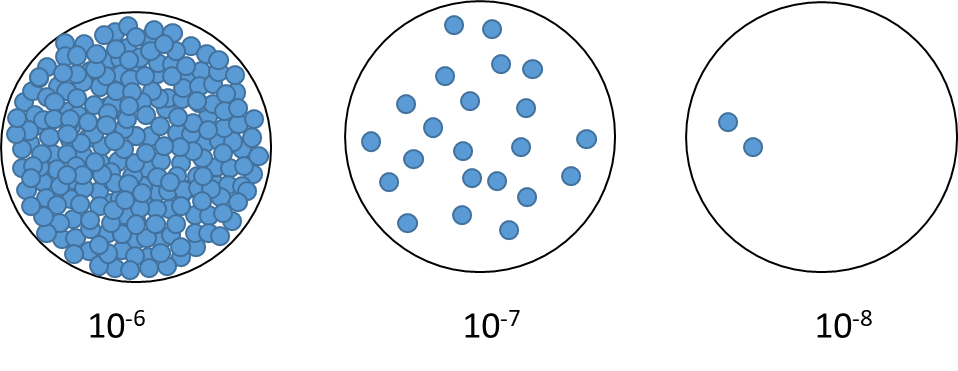
2. Practice Calculating Phage Titers

Calculate the titer (pfu/mL) for each line of data in this student’s notebook.

| **Phage Name** | **Sample Vol** | **Dilution** | **Number of pfu** | **Dilution Factor** | **Titer Calculation** |
| --- | --- | --- | --- | --- | --- |
| Snoopy | 10 µL | 10-3 | 45 | 1,000 | (45pfu/10 µL) X 1000 µL/1 mL) X 1,000 = 4.5 X 106 pfu/mL |
| RollTide | 10 µL | 10-2 | 168 | 100 | (186 pfu/10 µL) X (1000 µL/1 mL) X 100 = 1.68 X 106 pfu/mL |
| Hammy | 5 µL | 10-3 | 39 | 1,000 | (39 pfu/5 µL) X (1000 µL/1mL) X 1,000 = 7.8 X 106 pfu/mL |
| WarEagle | 10 µL | 10-6 | 132 | 1,000,000 | 132 pfu/10 µL) X (1000 µL/1mL) X 1,000,000 = 1.32 X 1010 pfu/mL |
| DeBarni | 5 µL | 10-5 | 101 | 100,000 | 101 pfu/5 µL X 1000 µL/1 mL) X 100,000 = 2.02 X 109 pfu/mL |

3.  Anita did 8 1:10 dilutions of her high titer lysate (HTL) and spotted 5 µL of each dilution on a plate with a lawn of host bacteria.    


Next, Anita did a full plate titer assay for the 10-6 through 10-8 dilutions. She plated 10 µL of diluted lysate on each plate. Below are the results of the serial dilutions that Anita plated.

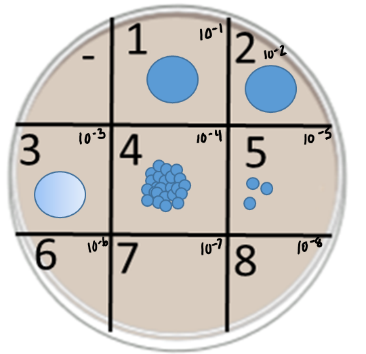


What is the titer of her HTL?  Show your work and include the units.

22 plaques/10 µL X 1000uL/1 mL X 107 = 2.2 X 1010 pfu/mL

Based on the information from the full plate titer assay, how many plaques do you estimate are present on the 10-6 spot on the titer assay spot test?

110 plaques. If there are 22 plaques on the 10-7 plate where 10 µL of lysate were added, then there are approximately 10 plaques on the 10-7 spot on the spot titer plate where 5µL of lysate were spotted. Therefore, there should be approximately 110 plaques on the 10-6 spot because the 10-6 lysate is 10 times more concentrated than the 10-7 lysate.

6.  Anita needed more phage lysate to isolate phage DNA. She flooded the 10-6 plate to make a new lysate and then isolated phage DNA from the new lysate and then did a spot titer experiment using the new lysate. A DNA isolation experiment works best if the HTL has a concentration of at least 109 pfu/mL.  Anita’s spot test is shown below (she used the same protocol as described in question 3).  Is her lysate concentrated enough for DNA isolation?  Explain your answer.   
  
  
Answer: No, it is likely not concentrated enough.  The 10-5 spot looks like it has 3 plaques, so we could estimate that the 10-4 has 30.  If so, the titer would be (30pfu/5 µL X 1000 µL/1 mL X 104 = 6.0 X 107 pfu/mL) even though this number may not be accurate, it is not on the magnitude of 109.