Measurement of viruses by end-point dilution assay
Tissue culture infective dose 50 (TCID50)

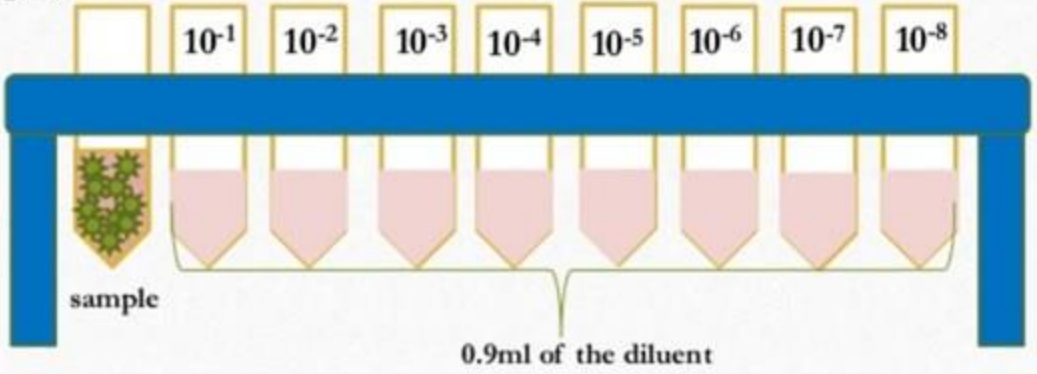
The end-point dilution assay was used to measure virus titer before the development of the plaque assay, and is still used for viruses that do not form plaques. Serial dilutions of a virus stock are prepared and inoculated onto replicate cell cultures, often in multi-well formats (e.g. 96 well plastic plates). The number of cell cultures that are infected is then determined for each virus dilution, usually by looking for cytopathic effect.

In an end-point dilution assay, 10 monolayer cell cultures were infected with each virus dilution. After an incubation period, plates that displayed cytopathic effects were scored with a +. At high dilutions, none of the cell cultures are infected because no particles are present. At low dilutions, every cell culture is infected. Half of the cell cultures showed cytopathic effects at the 10^{-5} dilution. This is the *end point*: the dilution of virus at which 50% of the cell cultures are infected. This number can be calculated from the data and expressed as 50% infectious dose (ID_{50}) per milliliter. The virus stock in this example contains 10^5 ID_{50} per ml.

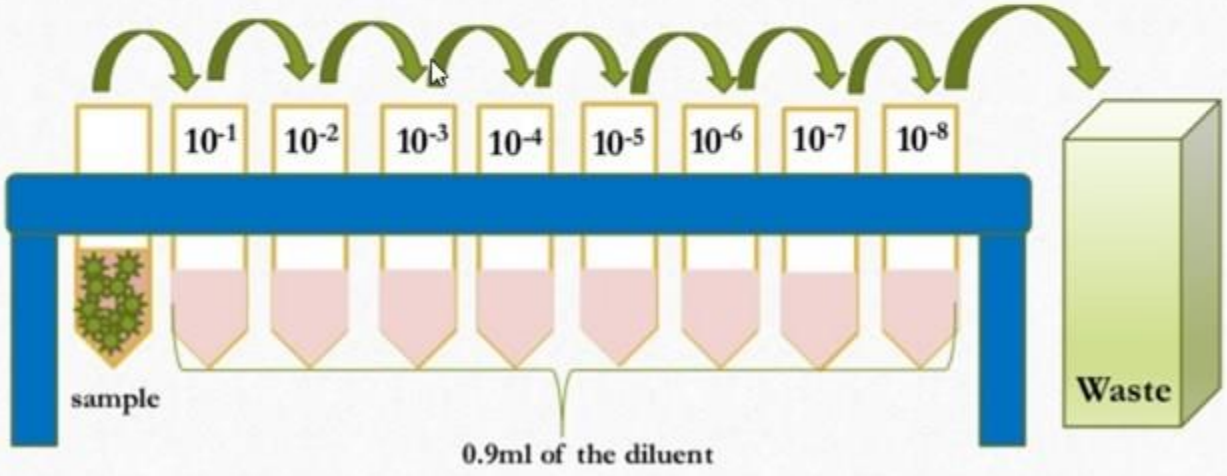
On the day of infection: Preparation of dilutions

10-fold serial

1. Set up a row of the appropriate number of eppendorf tubes in a rack.
2. Label with the dilutions to be used.
3. Pipette 0.9 ml of the diluent into the second and subsequent eppendorf.
4. Vortex virus sample, pipette the virus sample to be diluted into the first eppendorf (original undiluted sample).

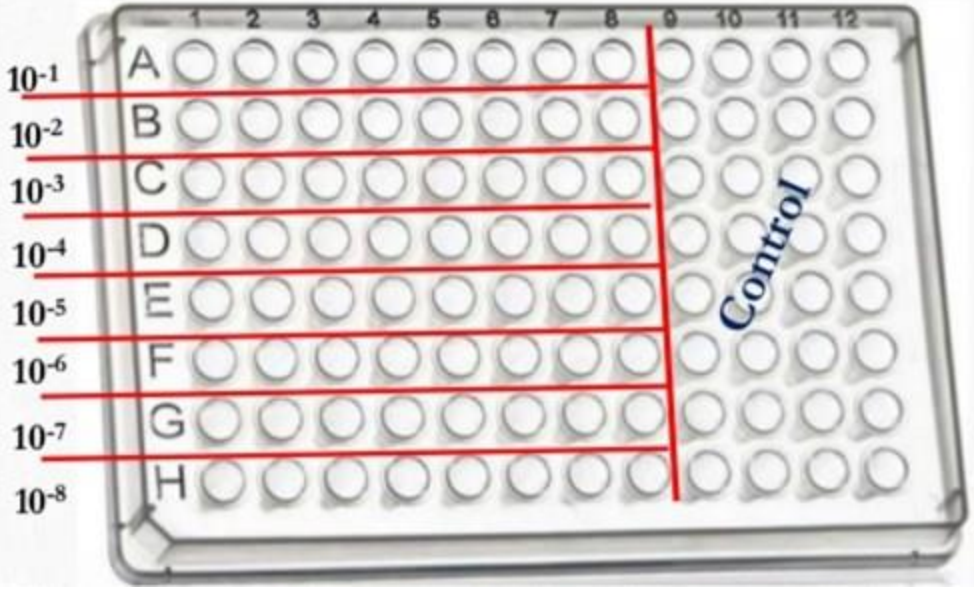


5. Transfer 0.1 ml from the first tube to the second.
6. Mix thoroughly but gently (10^{-1} dilution).
7. Transfer 0.1 ml of the volume of the second bijou to the third and mix as before (10^{-2} dilution).
8. Repeat this step to the end of the dilution series.
9. Discard 0.1ml from the last tube to the waste container



Additions of virus dilutions to cells:

1. Label the rows of 96-well plate by drawing grid lines for the dilution which will be plated.
2. If you are testing many virus samples, make sure you label each plate with the virus sample
3. Include negative wells on each plate which will not be infected.

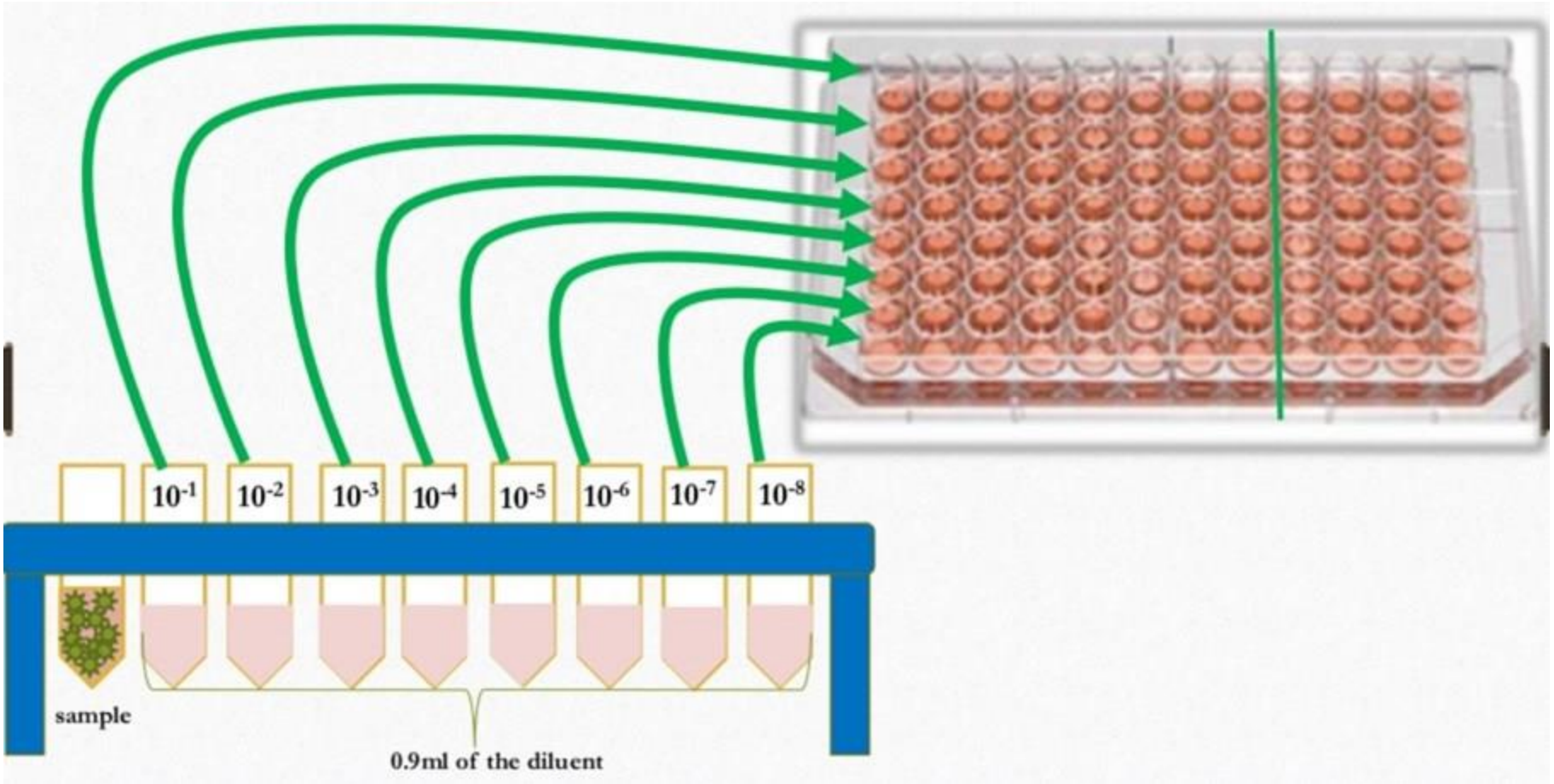


1. Remove all but 0.1 ml of media from each well by vacuum aspiration. (For influenza virus, monolayers should be rinsed once with serum free media before 0.1 ml infection media is added per well).

2. Starting from the most dilute sample, add 0.1 ml of virus dilution to each of the wells for that dilution.

3. Be careful not to track the pipette tip over the wells, and continue infecting the plate with 0.1 ml of virus dilution per well, infecting eight wells per dilution, proceeding backwards through the dilutions.

4. Change the pipette tip when necessary.


10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
10^{-7}
10^{-8}
sample
0.9ml of the diluent

5. Allow virus to adsorb to cells at 37° C for 2 hours or at a temperature indicated for the specific virus being titered (some viruses grow better at 34°C, including influenza).

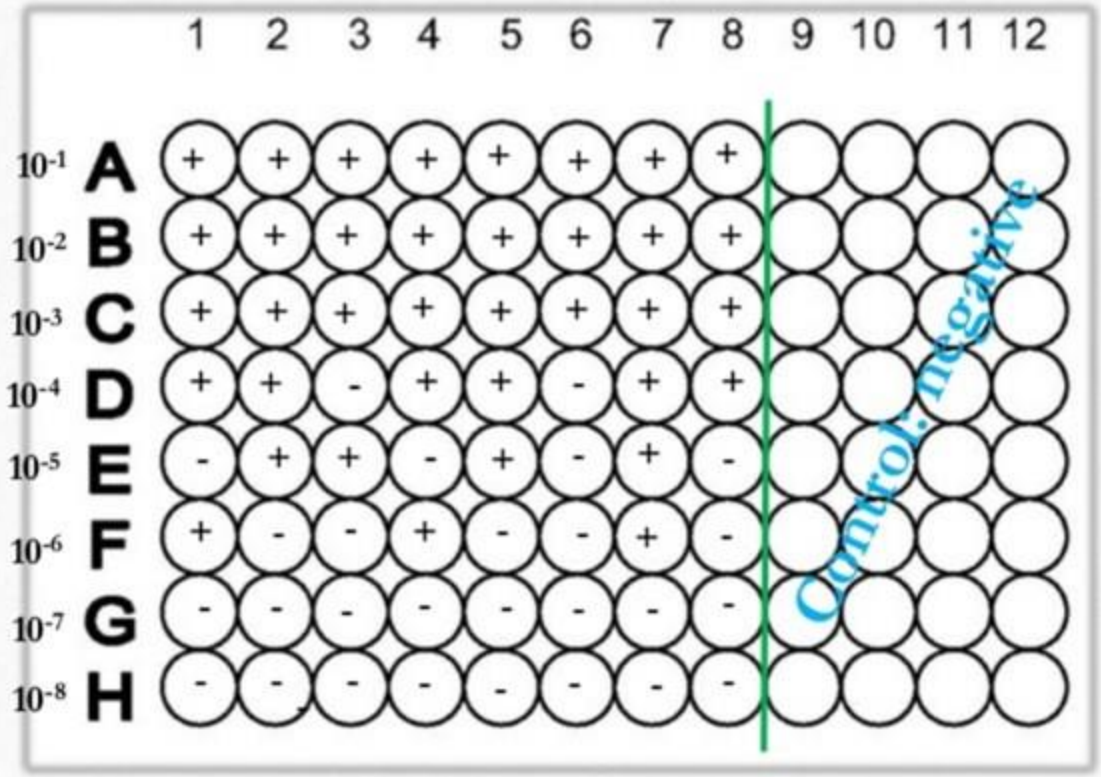
6. After adsorption, remove virus inoculum with vacuum, beginning by sucking the most dilute wells and proceeding backwards to less dilute. Alternatively, leave the virus on the cells.

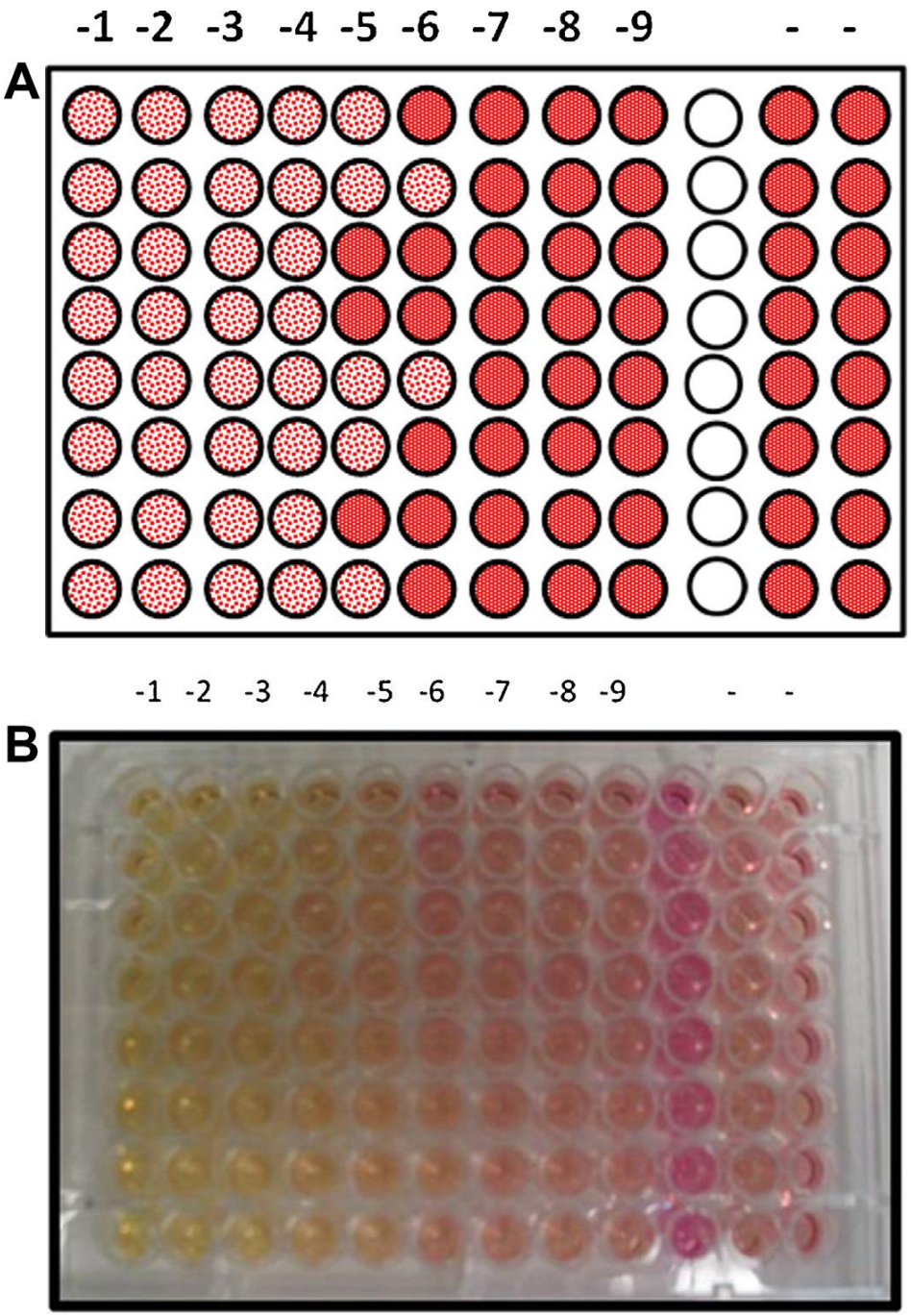
7. Add 0.5 ml Infection Medium (DMEM, 2% FBS, 4 mM Glutamine, 1X PSF) to each well. Do not touch the wells with the pipette. For influenza virus, add Infection Media without serum, with TPCK trypsin.

8. Place plates at 37oC or 34oC, depending upon instructions and monitor CPE using the inverted microscope over a period of one to four weeks, depending on the cultural characteristics of the virus in question. Record the number of positive and negative wells.

TCID 50 Calculation

Example:

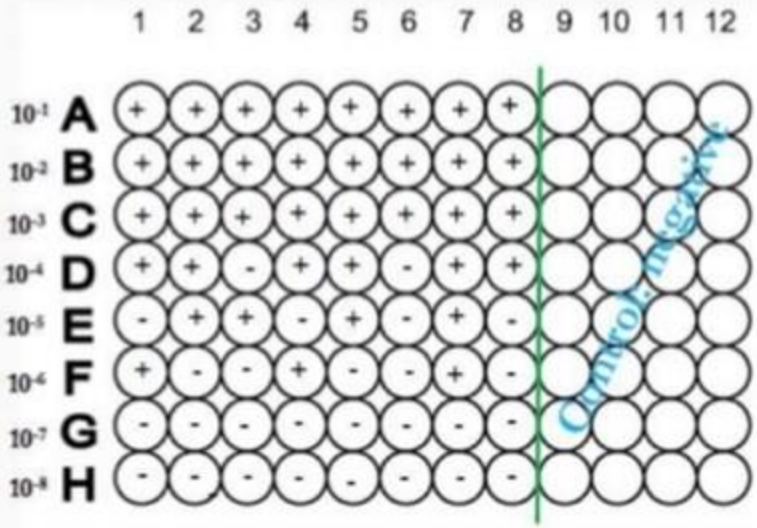



-1 -2 -3 -4 -5 -6 -7 -8 -9 - -
A
-1 -2 -3 -4 -5 -6 -7 -8 -9 - -
B

Reed and Muench method

Cumulative

Dilution	Alive	Dead	A	B	A+B	A / A+B	%
10^{-1}	8	0	37	0	37	1	100
10^{-2}	8	0	29	0	29	1	100
10^{-3}	8	0	21	0	21	1	100
10^{-4}	6	2	13	2	15	0.87	87
10^{-5}	4	4	7	6	13	0.54	54
10^{-6}	3	5	3	11	14	0.21	21
10^{-7}	0	8	0	19	19	0	0
10^{-8}	0	8	0	27	27	0	0



Difference of logarithms = $\frac{\text{(mortality at dilution next above 50\%)-50\%}}{\text{(mortality next above 50\%)-(mortality next below 50\%)}} = \frac{54-50}{54-21} = 0.12$

$TCID_{50}$= log of lower dilution (immediately above 50%) + (prof. distance x log. of dilution factor) = 5 + (0.12 x 1) = 5.12

Therefore, A dilution of $10^{5.12}$ contains 1 $TCID_{50}$ (same for LD_{50})

Thee are on-line TCID50 calculators where you enter the values in Excel sheet and TCID50 will be calculated

Spearman-Karber method

- log10 50% end point dilution = $- (x0 - d/2 + d \sum ri/ni)$

x0 = log10 of the reciprocal of the highest dilution (lowest concentration) at which all wells are positive

d = log10 of the dilution factor

ni = number of wells used in each individual dilution

ri = number of positive wells (out of ni).

Summation is started at dilution x0.

From this example:

x0 = 3 d = 1 ni = 8 $\sum ri = 21$

log10 50% end point dilution = $- (3 - 0.5 + (21/8) = 5.13 = 10^{5.13}$

Dilution	Alive	Dead	A	B	A+B	A / A+B	%
10^{-1}	8	0	37	0	37	1	100
10^{-2}	8	0	29	0	29	1	100
10^{-3}	8	0	21	0	21	1	100
10^{-4}	6	2	13	2	15	0.87	87
10^{-5}	4	4	7	6	13	0.54	54
10^{-6}	3	5	3	11	14	0.21	21
10^{-7}	0	8	0	19	19	0	0
10^{-8}	0	8	0	27	27	0	0

Newly proposed method (Ramakrishnan MA. Determination of 50% endpoint titer using a simple formula. World J Virol. 2016 May 12;5(2):85-6. doi: 10.5501/wjv.v5.i2.85)

- log10 50% end point dilution = -[(total number of positive wells/number of wells inoculated per dilution) + 0.5] × log dilution factor.

From this example:

log10 50% end point dilution = (37/8) +0.5 = 5.13 = $10^{5.13}$

Dilution	Alive	Dead	A	B	A+B	A / A+B	%
10^{-1}	8	0	37	0	37	1	100
10^{-2}	8	0	29	0	29	1	100
10^{-3}	8	0	21	0	21	1	100
10^{-4}	6	2	13	2	15	0.87	87
10^{-5}	4	4	7	6	13	0.54	54
10^{-6}	3	5	3	11	14	0.21	21
10^{-7}	0	8	0	19	19	0	0
10^{-8}	0	8	0	27	27	0	0