

# Survival of Shiga toxin-producing *E. coli* in veal

Data statistical analysis

Kyriaki

# Project description

- Food Microbiology
- PhD student needs assistance in data analysis
- 2-month long project

## Main problem:

- ANOVA did not work but graphs of data show no problem

# Project description

1. Design the project / write the protocol
2. Predict the duration
3. Execute (n=3)
4. Data analysis

(if  $P_{trial} < 0.05$ , we will have to repeat it once again)

Problems come up, ask for statistical assistance

# Protocol

## Preparation:

- Grow cells

## Beginning:

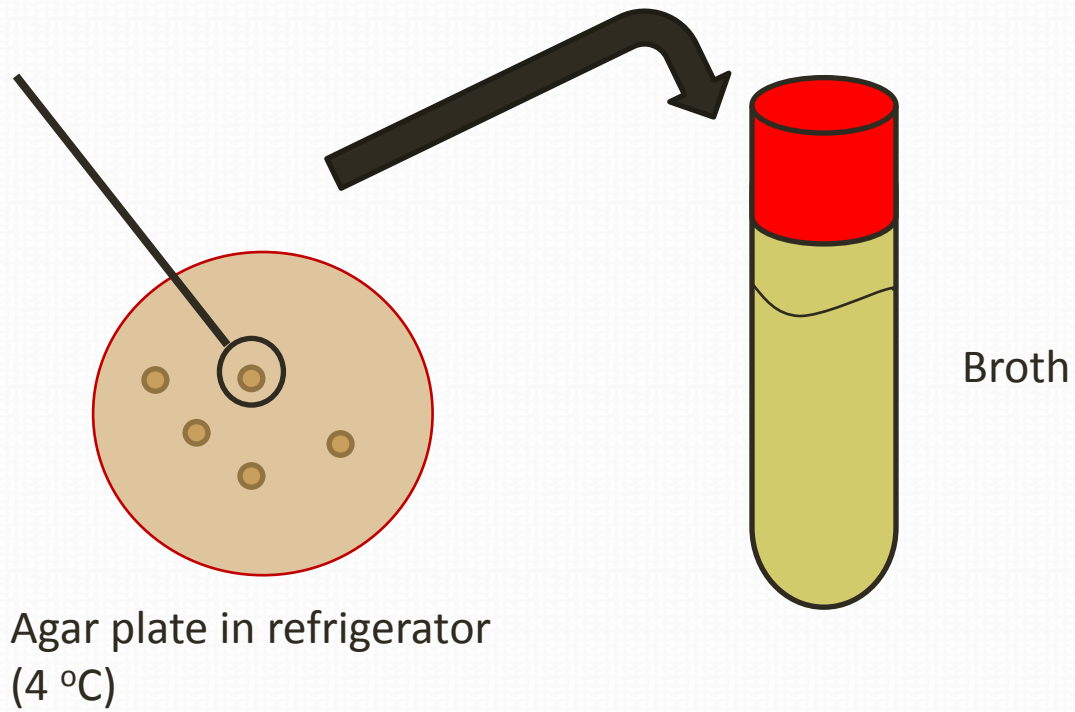
- Transferring cells on veal samples.
- Plating

## Any subsequent day:

- Plating

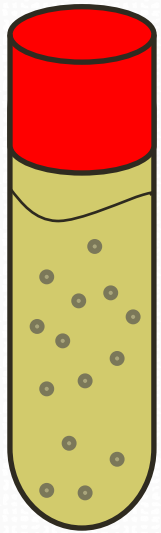
# Protocol

## Preparation:



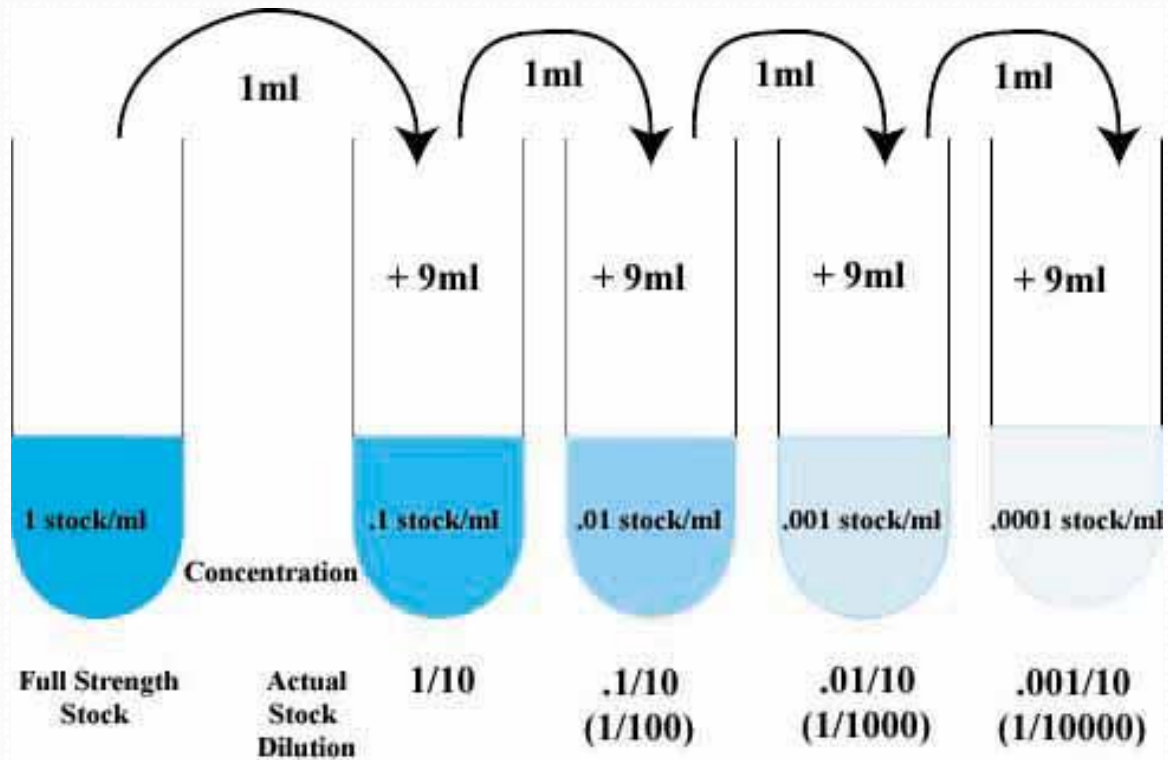
# Protocol

Beginning:



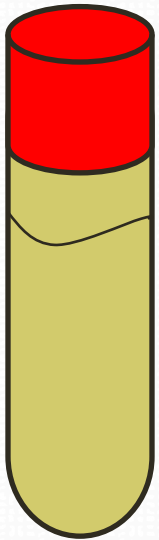
Inoculated  
BHIB

$10^9$  cells/ml



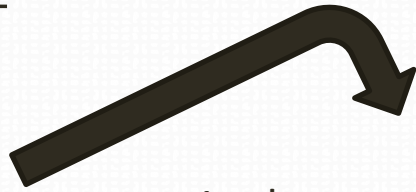
# Protocol

Beginning:

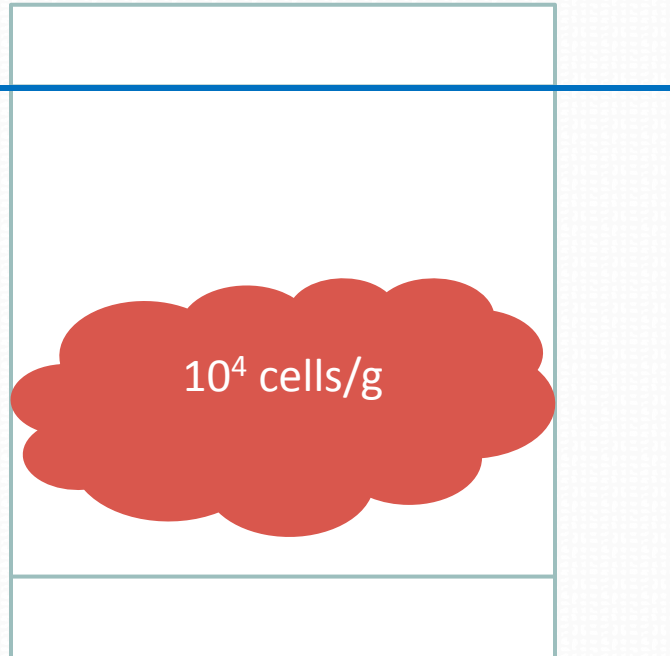


Inoculated  
Broth

$10^5$  cells/ml



1 ml

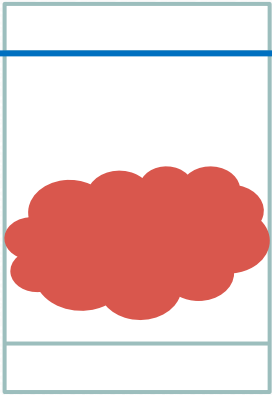


$10^4$  cells/g

# Protocol

Stored at 10 °C

time 0



time 18



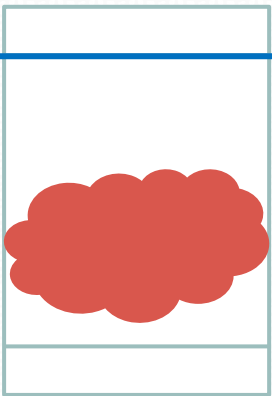
time 24



time 36



time 48



time 72



time 96



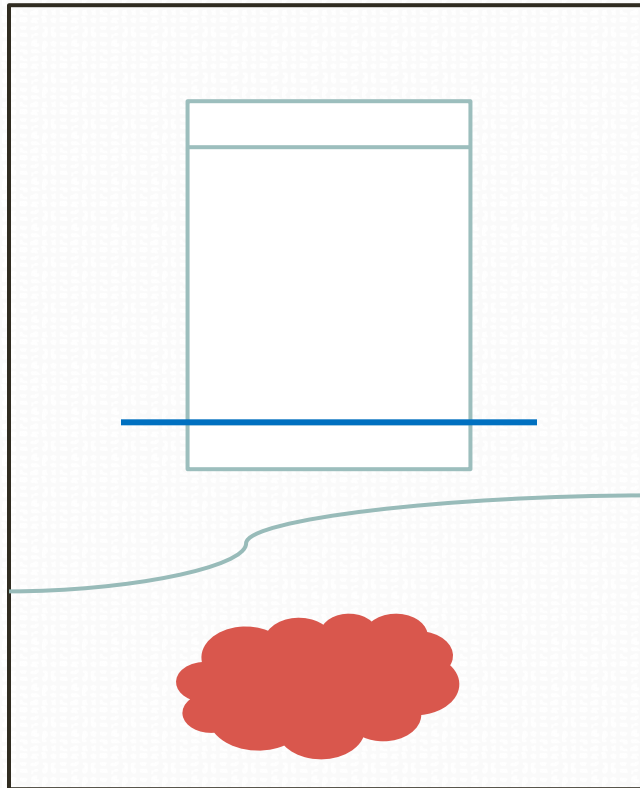
time 120





# Protocol

## Plating



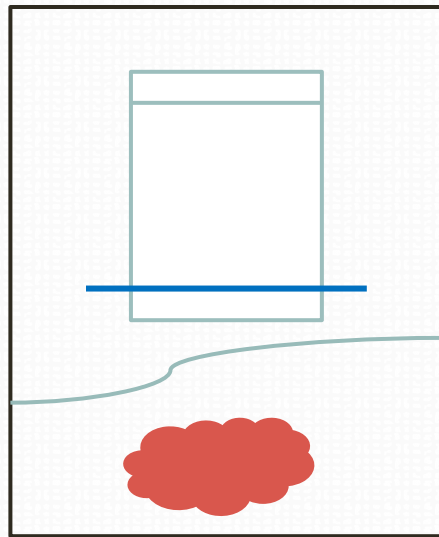
25 g of veal  
+  
99 ml diluent  
+  
1 ml of inoculum

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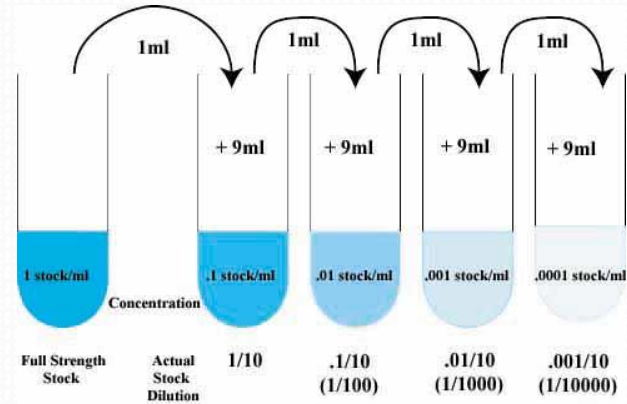
Total 125  
ml or g

# Protocol

## Plating (continued..)

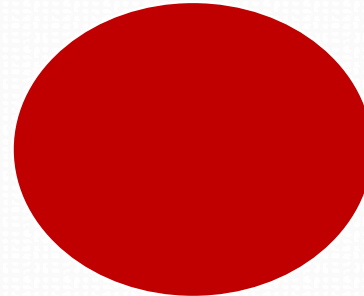


1 ml  
→



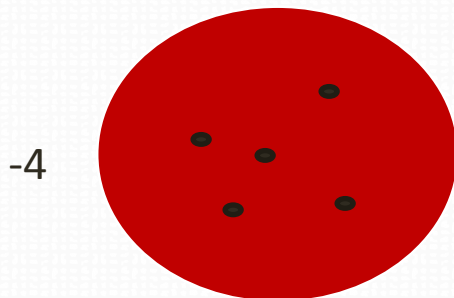
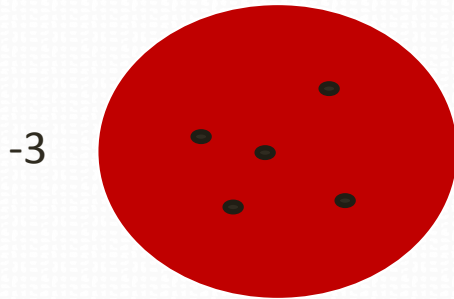
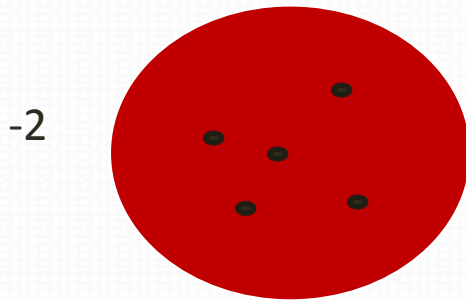
0.1 ml

agar plate



# Protocol

## Data acquisition



1. Record the count number (25-250 colonies)
2. Calculate the log number (formula)
3. Statistical analysis

# Data analysis

strain	time	cfu/g	logs	delta-logs	trial	week
O157	0	6105.769	3.78574	0	1	2
O157	18	4951.923	3.694774	-0.0909665	1	2
O157	24	4759.615	3.677572	-0.1081685	1	2
O157	36	4951.923	3.694774	-0.0909665	1	2
O157	48	4375	3.640978	-0.1447623	1	2
O157	72	3942.308	3.595751	-0.1899899	1	2
O157	96	4278.846	3.631327	-0.1544137	1	2
O157	120	3942.308	3.595751	-0.1899899	1	2
O157	0	4711.538	3.673163	0	2	6
O157	18	4086.538	3.611356	-0.0618071	2	6
O157	24	3942.308	3.595751	-0.0774122	2	6
O157	36	4423.077	3.645724	-0.0274382	2	6
O157	48	4134.615	3.616435	-0.0567276	2	6
O157	72	4759.615	3.677572	0.0044091	2	6
O157	96	3750	3.574031	-0.0991315	2	6
O157	0	6490.385	3.81227	0	3	8
O157	18	5576.923	3.746395	-0.0658758	3	8
O157	24	5096.154	3.707243	-0.1050279	3	8
O157	36	4951.923	3.694774	-0.1174965	3	8
O157	48	4807.692	3.681937	-0.1303338	3	8
O157	72	5769.231	3.761118	-0.0511525	3	8
O157	96	5144.231	3.71132	-0.10095	3	8
O157	0	3413.462	3.533195	0	4	4
O157	18	3509.615	3.54526	0.0120645	4	4
O157	24	4182.692	3.621456	0.0882609	4	4
O157	36	2980.769	3.474328	-0.0588667	4	4
O157	48	3221.154	3.508011	-0.0251835	4	4
O157	72	2451.923	3.389507	-0.1436882	4	4
O157	96	1875	3.273001	-0.2601937	4	4
O157	120	2500	3.39794	-0.135255	4	4
O145	0	2067.308	3.315405	0	1	2
O145	18	1490.385	3.173298	-0.1421068	1	2
O145	24	1875	3.273001	-0.0424038	1	2
O145	36	1682.692	3.226005	-0.0894004	1	2
O145	48	2019.231	3.305186	-0.0102192	1	2



# Data analysis

Time (h)	Dilution	Correct dilution	Count	Dilution factor	CFU/g	Log CFU/g	Delta-log CFU/g
0	-2	-1	77	0.208	3701.923077	3.56842739	0
18	-2	-1	113	0.208	5432.692308	3.735015109	0.166587718
24	-2	-1	112	0.208	5384.615385	3.731154688	0.162727297
36	-2	-1	79	0.208	3798.076923	3.579563756	0.011136366
48	-2	-1	80	0.208	3846.153846	3.585026652	0.016599262
72	-2	-1	117	0.208	5625	3.750122527	0.181695137
96	-2	-1	101	0.208	4855.769231	3.686258039	0.117830649
120	-2	-1	64	0.208	3076.923077	3.488116639	-0.080310751

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**Correct dilution** is one number less, because we plated 0.1ml of the cell population, so a 10-fold dilution is made into the pipette.

**Dilution factor** is 26/125, because we used 25g of veal inoculated with 1ml of bacteria (we assume this as another 1g) and then 99ml of diluent were added before plating, so total is 125.

# Data analysis

Time (h)	Dilution	Correct dilution	Count	Dilution factor	CFU/g	Log CFU/g	Delta-log CFU/g
0	-2	-1	77	0.208	3701.923077	3.56842739	0
18	-2	-1	113	0.208	5432.692308	3.735015109	0.166587718
24	-2	-1	112	0.208	5384.615385	3.731154688	0.162727297
36	-2	-1	79	0.208	3798.076923	3.579563756	0.011136366
48	-2	-1	80	0.208	3846.153846	3.585026652	0.016599262
72	-2	-1	117	0.208	5625	3.750122527	0.181695137
96	-2	-1	101	0.208	4855.769231	3.686258039	0.117830649
120	-2	-1	64	0.208	3076.923077	3.488116639	-0.080310751

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$$\text{CFU/g} = \frac{\frac{\text{count}}{\text{dilution factor}}}{10^{\text{correct dilution}}}$$

**Delta-log** is actually the log of the ratio of the final value to the initial value, but presented here as subtraction of these 2 values.

## Questions to be answered:

- Is there a need for another repeat?
- Are the strains significantly different from one another?
- Does time affect significantly the survival of each strain?

Any other question you are curious about (i.e. formula, week-trial etc.)