Quantifying the bias of the viable cell enumeration process and its impact on microbial inactivation

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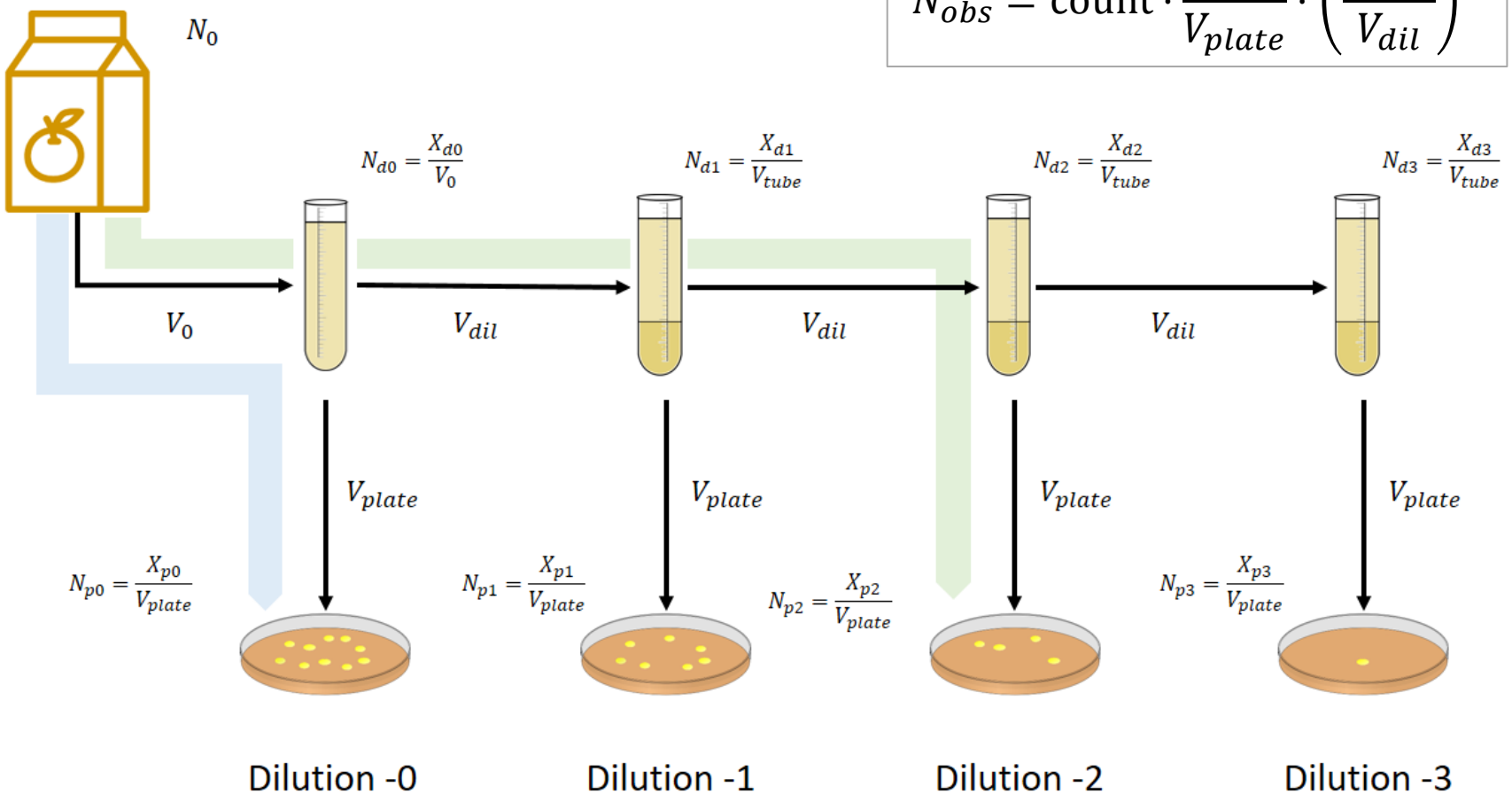
Serial dilution/plate count

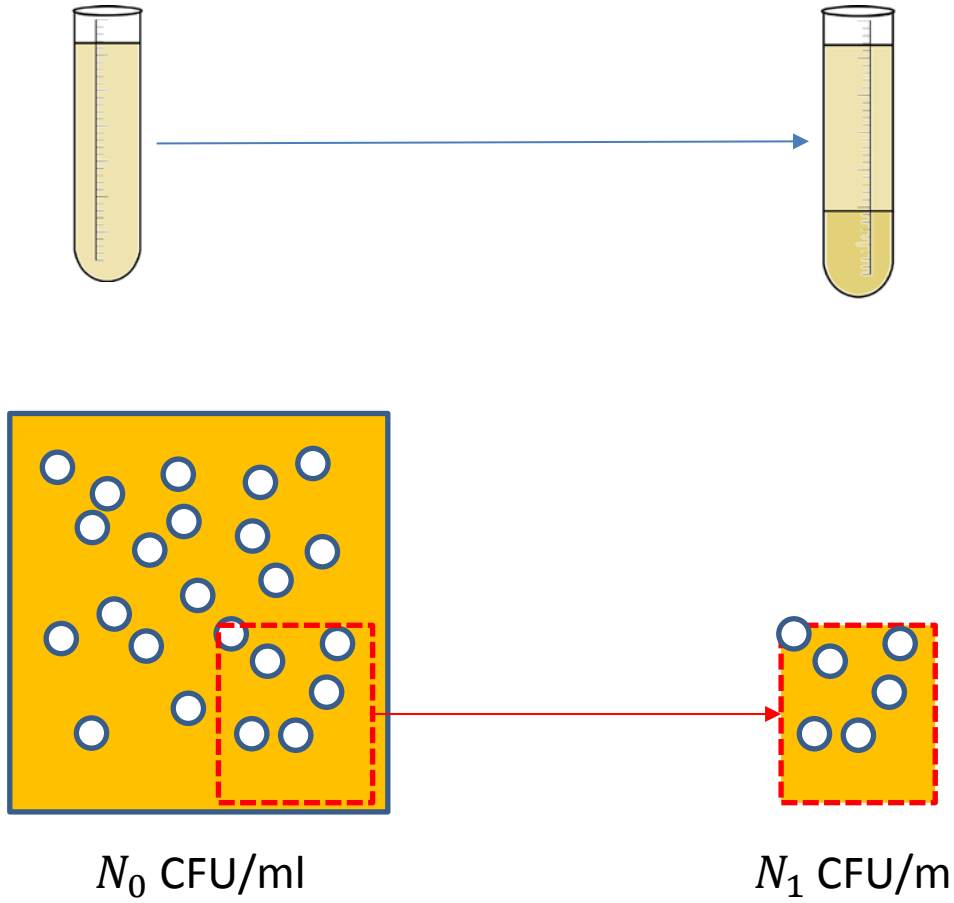
$$N_{obs} = \text{count} \cdot \frac{1}{V_{plate}} \cdot \left(\frac{V_{tube}}{V_{dil}}\right)^{n}$$



Sources of uncertainty

- (Sub)sampling error.
- Log-transformation.
- Errors in volumes (calibration errors).
- Counting errors.
- Clustering of bacterial cells.
- …

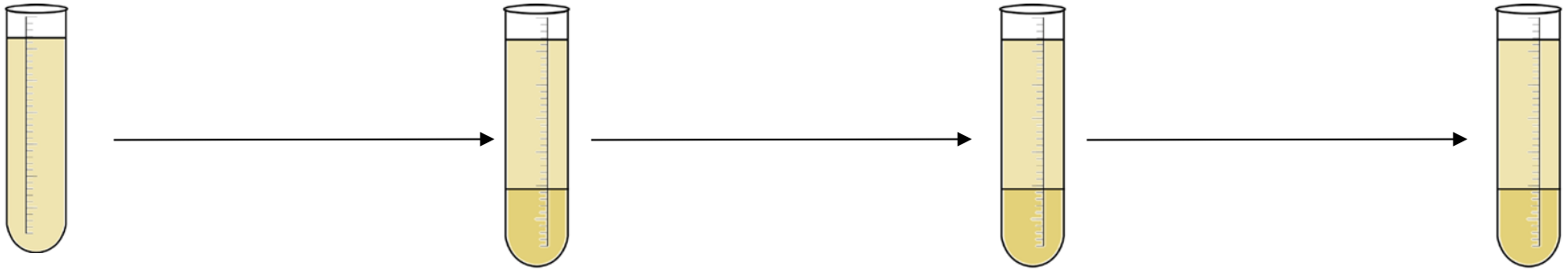
Modelling of one (sub)sampling



- Finite volumes.
- Finite number of particles.
- Uncorrelated positions (no clumps; perfect mixing).

$$N_1 \sim Binom(N_0 \cdot V_0, p = \frac{V_1}{V_0})$$

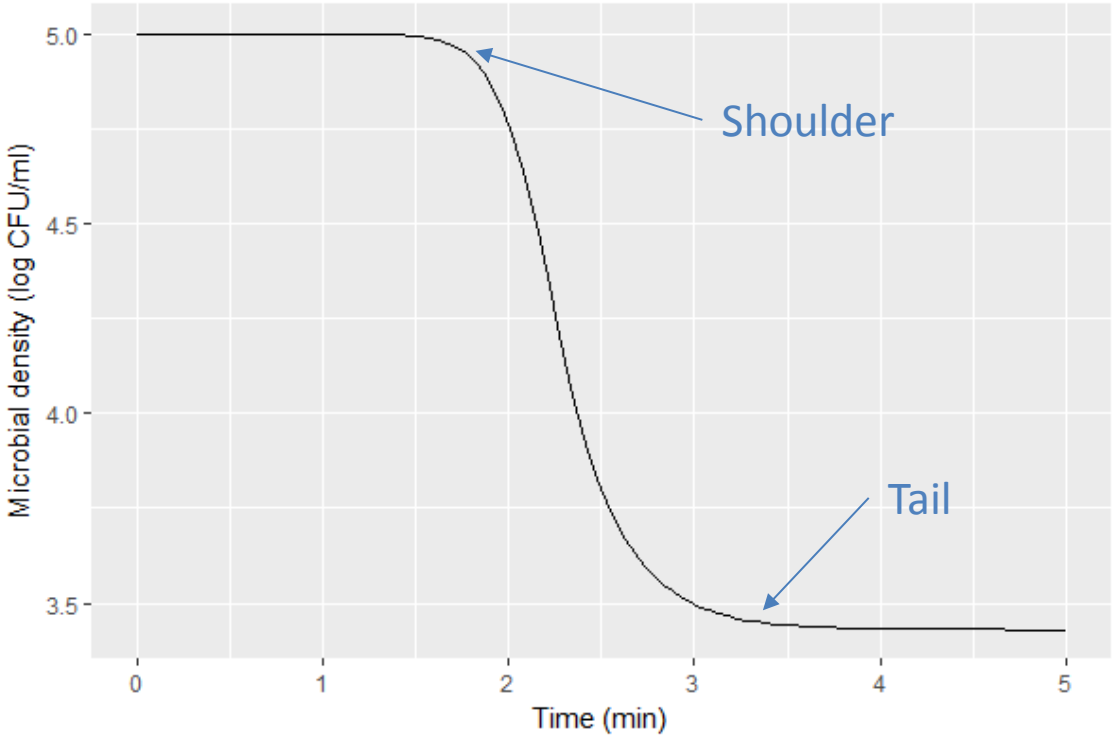
Modelling of serial dilutions



$$N_i|N_{i-1} \sim Binom(N_{i-1} \cdot V_{i-1}, p = \frac{V_i}{V_{i-1}})$$

$$N_n|N_0 \sim Binom(N_0 \cdot V_0, p = \frac{V_n}{V_0} = f^n)$$

Case study: microbial inactivation



Survivor curve typically non-lineal

Shoulder: Se debe de mantener el tratamiento para inactivar

Tail: More resistant fraction/acclimation.

Very relevant for food safety

How are non-linearities affected by experimental error?

Simulation of inactivation experiments

1. Log-linear survivor curve $\log N(t) = \log N_0 - \frac{1}{D}t$

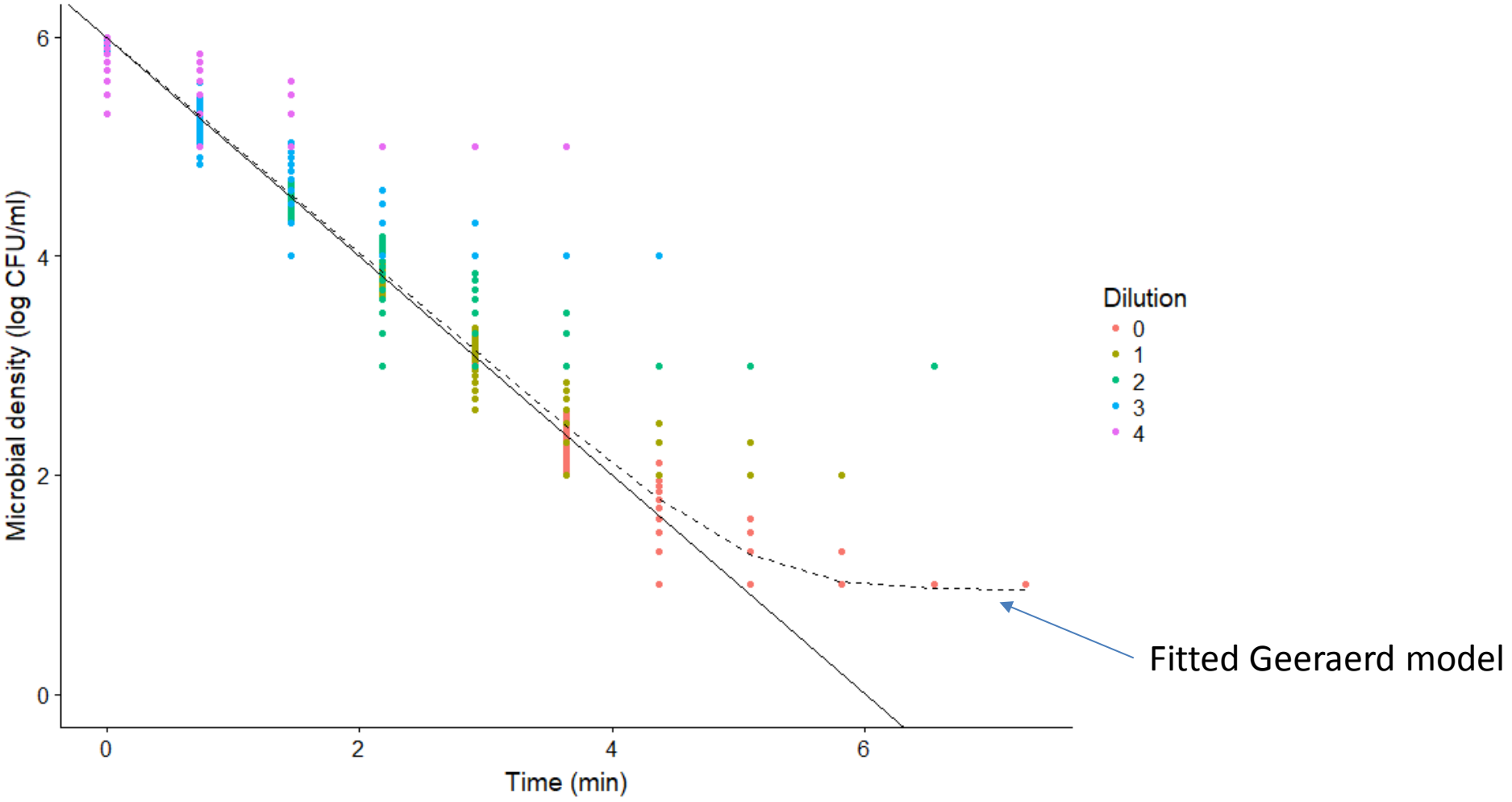
2. 11 sampling points. Dilutions 0 to -5.

3. Plate count according to Binomial distribution.

4. Plates with >300 colonies omitted.

5. 600 experiments simulated.

Uncertainty can cause bias



Experimental design affects bias

	0.1 mL en placa	0.5 mL en placa	1 mL en placa
0 dilutions	0.87 log CFU/ml	0.17 log CFU/ml	-0.17 log CFU/ml
1 dilution	1.86 log CFU/ml	1.17 log CFU/ml	0.84 log CFU/ml
2 dilutions	2.89 log CFU/ml	2.17 log CFU/ml	1.88 log CFU/ml
3 dilutions	3.94 log CFU/ml	3.22 log CFU/ml	2.92 log CFU/ml
4 dilutions	5.00 log CFU/ml	4.25 log CFU/ml	3.93 log CFU/ml

Conclusions/outlook

- Uncertainty related to experimental design.
- Uncertainty can cause bias.
- Reporting of volumes plated/dilutions in scientific publications.
- Do not mix different dilutions/volumes plated.
- Detection limit (Chik et al. 2018, *Learning Something From Nothing: The Critical Importance of Rethinking Microbial Non-detects*. Frontiers in Microbiology).

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Tail or artefact? Illustration of the impact that uncertainty of the serial dilution and cell enumeration methods has on microbial inactivation

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Danke schön!

¡Muchas gracias!

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