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# Spotting method as a high throughput alternative to the conventional spread plating method for bacterial time-kill experiments.

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## Introduction

*In vitro* time-kill experiments are commonly performed to obtain information about the dynamics of a drug antimicrobial activity. It is however labor intensive due to the conventional spread plating method used to count bacteria. In this work we compare a spotting method with the conventional spread plating method.

## Materials and Methods

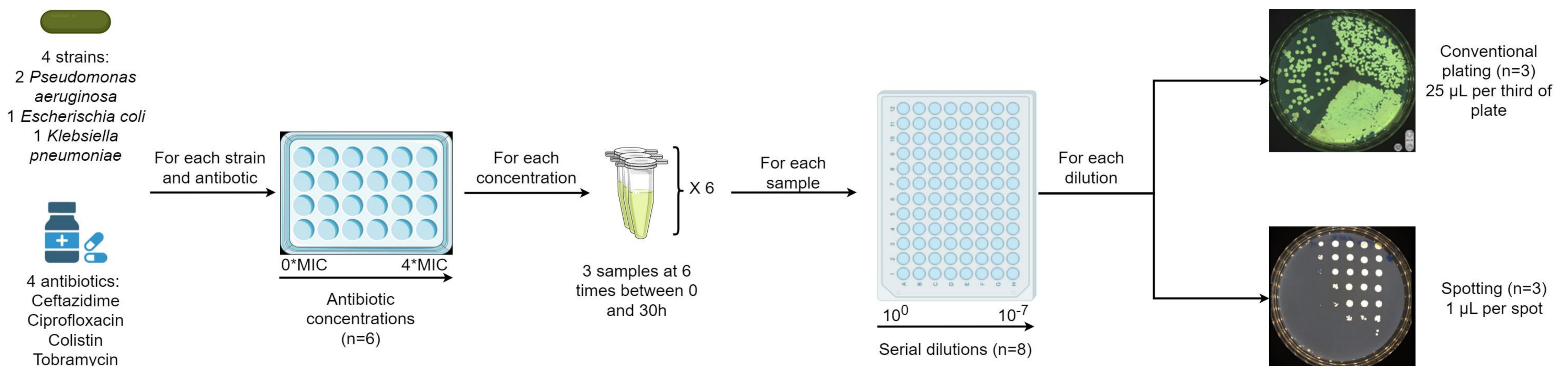


Figure 1: Experimental protocol.

### Correction of colony counts to account for overcrowding

- Colony forming unit counts were converted to bacterial densities after correcting for overcrowding (Figure 2) by the « most probable number » method from Martini *et al.* (1)
- Under this method, for a given measurement, the probability of overcrowding is higher when the number of counted colonies (Figure 3) is close to the maximal number of countable colonies.

### Validation of the spotting method

- To validate the spotting method against the conventional plating method we applied correlated bivariate least square regression developed by Francq and Govaerts (2) on  $\log_{10}$  transformed bacterial densities after exclusion of measurements below  $10^3$  CFU/mL (limit of detection of the spotting method)

### Mathematical methods and software

- Optimization of the likelihood function of the « most probable number » (1) method was performed using the R function `optim` (3). Correlated bivariate least square regression was performed using the `BivRegBLS` package (4). Data wrangling and graphing was performed with the `tidyverse` package (5)

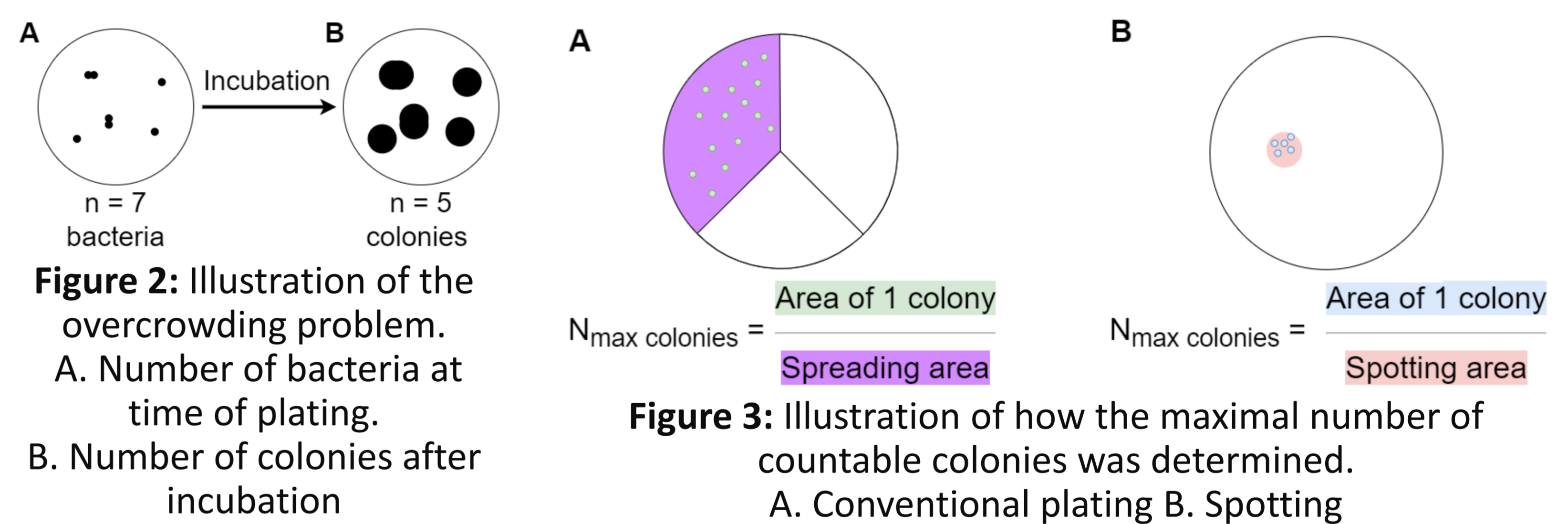


Figure 2: Illustration of the overcrowding problem.  
A. Number of bacteria at time of plating.  
B. Number of colonies after incubation

Figure 3: Illustration of how the maximal number of countable colonies was determined.  
A. Conventional plating B. Spotting

## Results

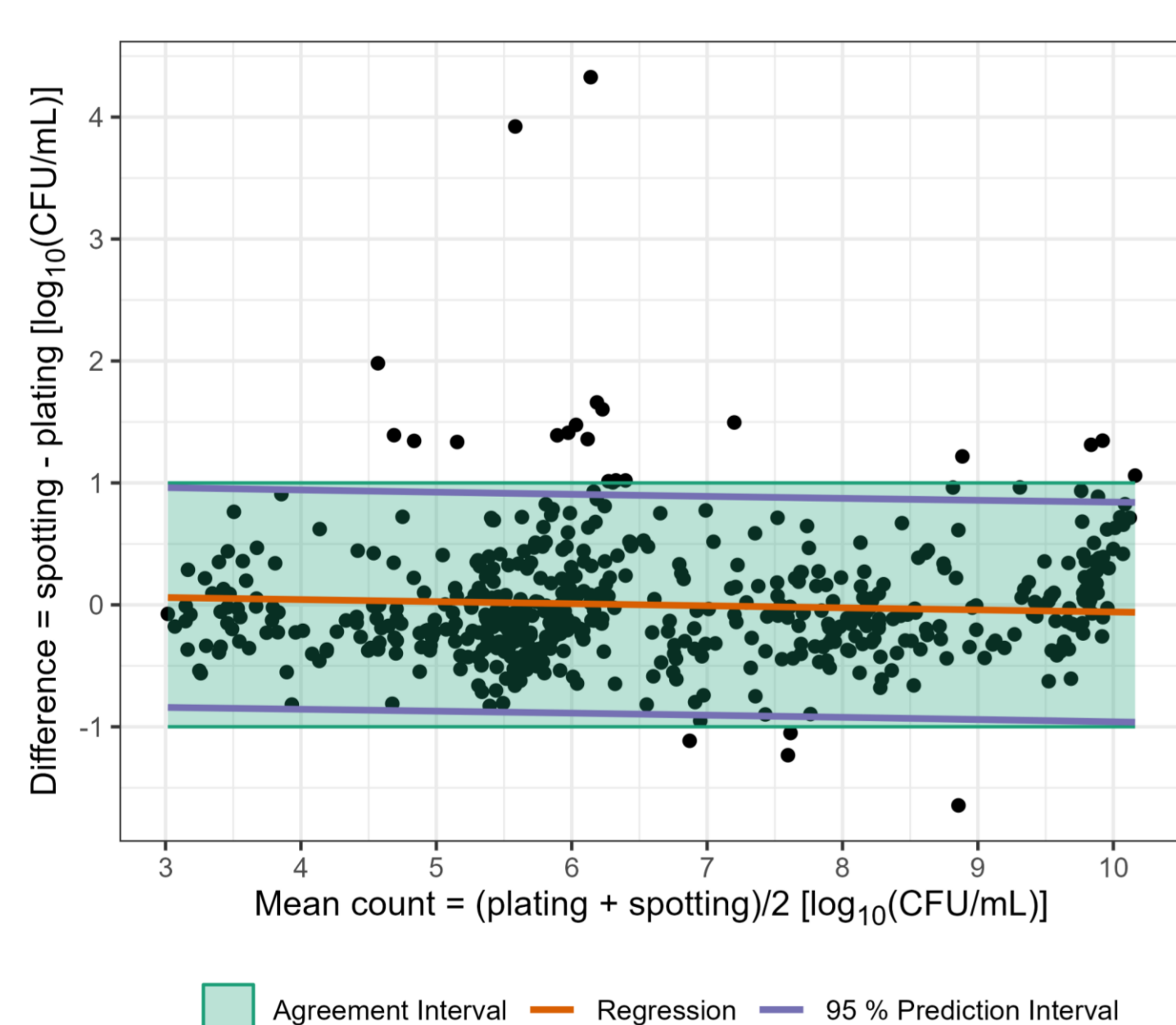


Figure 4: Bland-Altman plot (n=551).

Table 1: Performance of the two plating methods

	Conventional plating	Spotting
Number of time-kill per week	8-12	48-96
Mean maximal number of colonies per plating	675	7.84
Limit of detection (CFU/mL)	$4 \cdot 10^1$	$10^3$
Average bias ( $\log_{10}$ CFU/mL)		$6.6 \cdot 10^{-4}$
Repeatability standard deviation ( $\log_{10}$ CFU/mL)	0.105	0.445

- As seen in Table 1 spotting method was accurate but exhibited lower precision than the conventional plating method.
- The accuracy was confirmed over the whole range of measurements as evidenced by the red regression line on Figure 4.
- The two measurement methods were considered in agreement when the difference was between -1 and +1  $\log_{10}$  CFU/mL (Figure 4 green area). In our experiment they agreed since the 95% prediction interval of the differences (Figure 4 purple lines) lied inside the agreement interval (Figure 4 green area).
- This agreement is qualitatively shown by Figure 5 where typical time-kill profiles obtained by plating (Figure 5A) and by spotting (Figure 5B) are comparable.

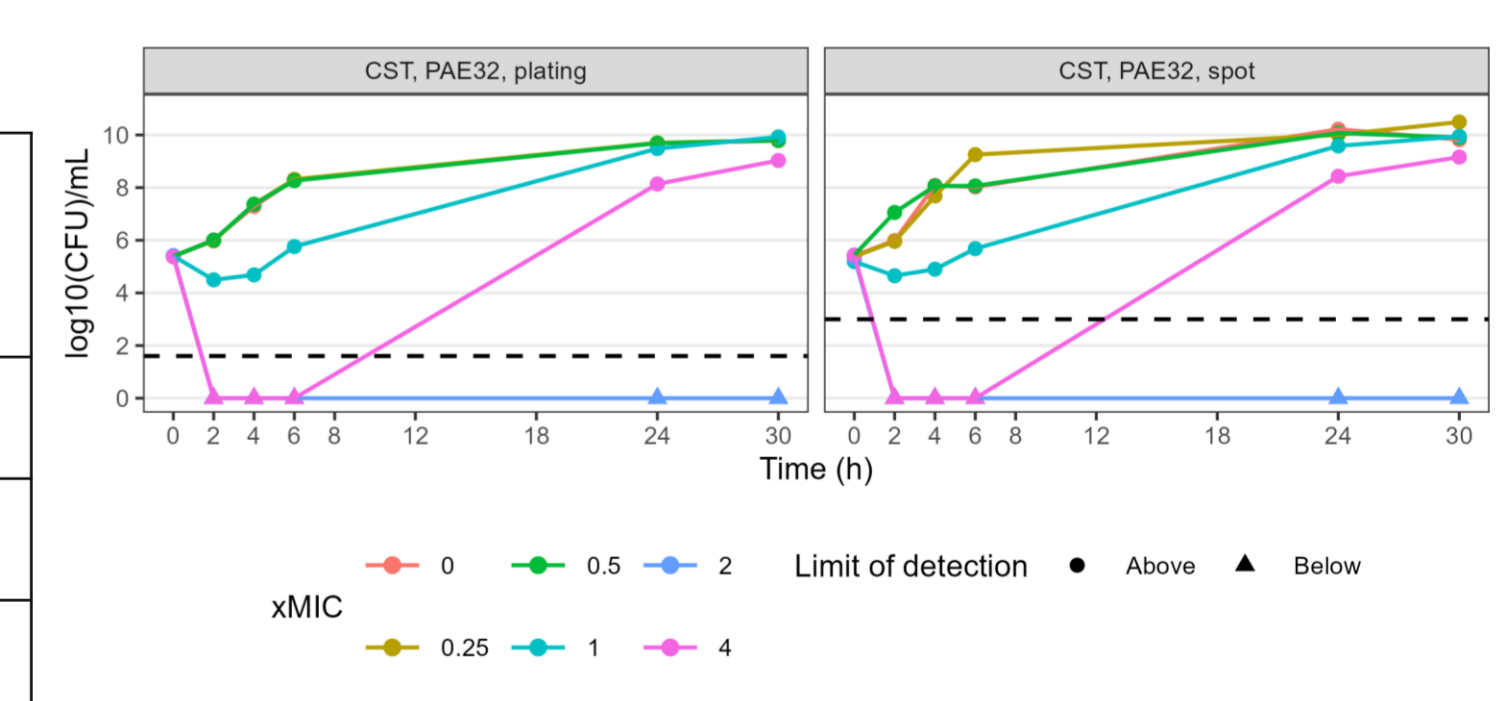


Figure 5: Typical time-kill results. A. Conventional plating B. Spotting

## Conclusion

The spotting method yields bacterial counting results comparable with the conventional plating method while being significantly higher throughput.

### References

- Martini *et al.* [10.1101/2023.05.18.541301](https://doi.org/10.1101/2023.05.18.541301)
- Francq and Govaerts, [10.1002/sim.6872](https://doi.org/10.1002/sim.6872)
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- Francq *et al.* [10.1002/sim.8709](https://doi.org/10.1002/sim.8709)
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