P2490

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

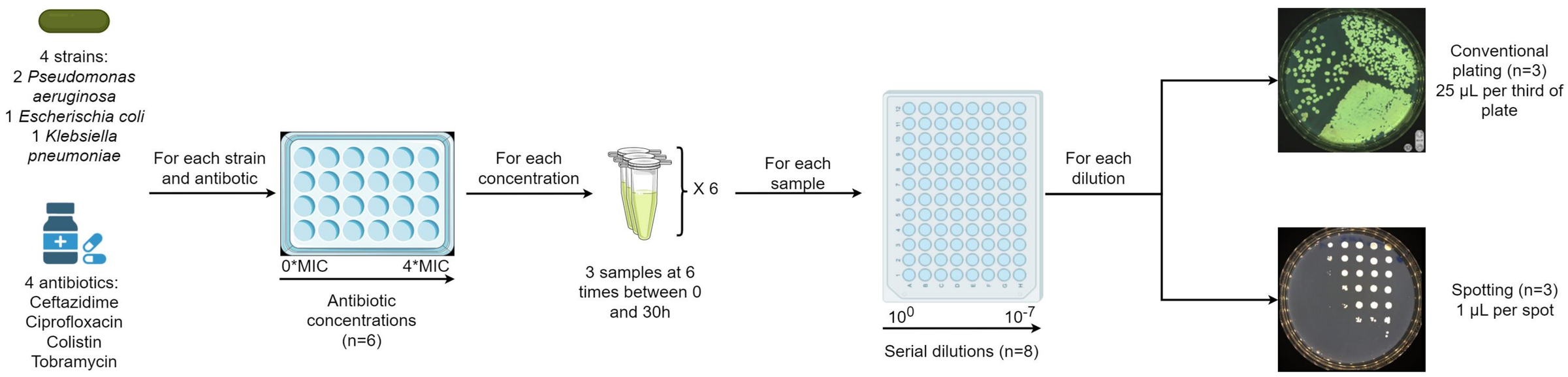

Figure 1: Experimental protocol.

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.

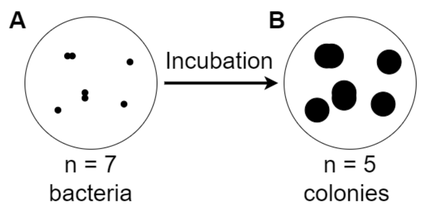

Figure 2: Illustration of the overcrowding problem.
A. Number of bacteria at time of plating.
B. Number of colonies after incubation

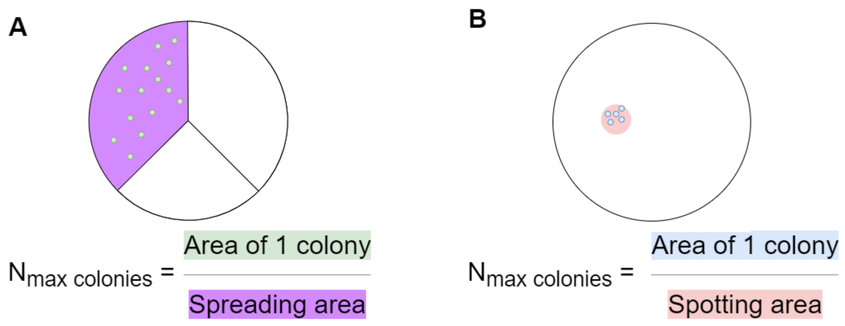

Figure 3: Illustration of how the maximal number of countable colonies was determined.
A. Conventional plating B. Spotting

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)

Results

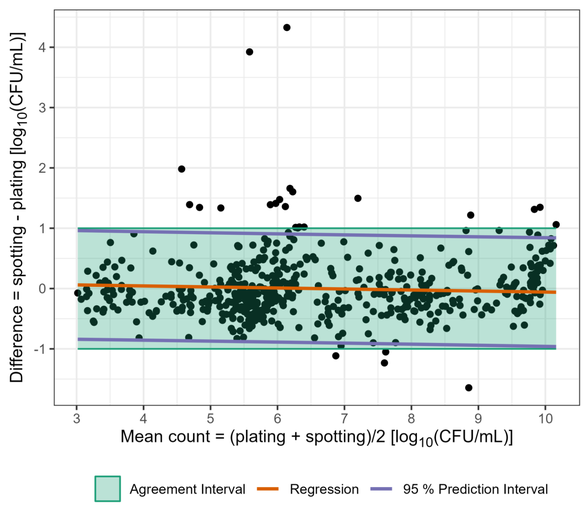

Figure 4: Bland-Altman plot (n=551).

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)	$4*10^1$	10^3
Average bias ($\log_{10}$ CFU/mL)		$6.6 * 10^{-4}$
Repeatability standard deviation ($\log_{10}$ CFU/mL)	0.105	0.445

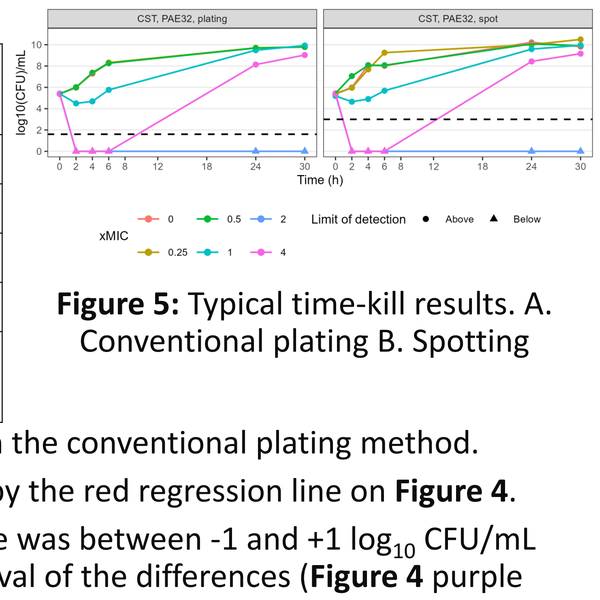

Figure 5: Typical time-kill results. A. Conventional plating B. Spotting

- 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.

Conclusion

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

References

1) Martini et al. 10.1101/2023.05.18.541301
2) Francq and Govaerts, 10.1002/sim.6872
3) R Core Team (2023) www.R-project.org
4) Francq et al. 10.1002/sim.8709
5) Wickham et al. 10.21105/joss.01686

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