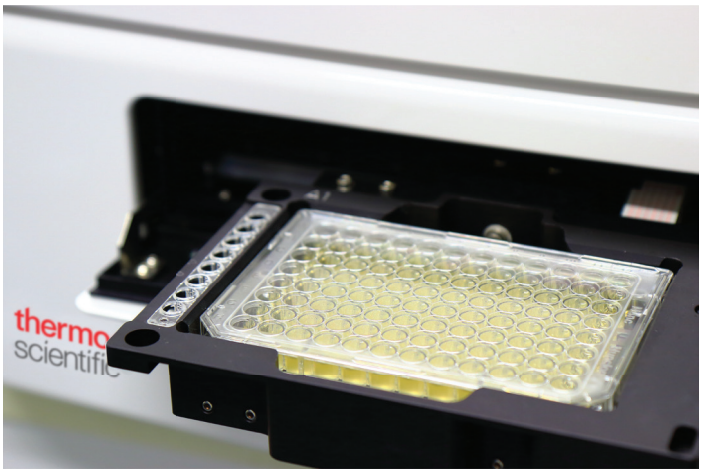
Following bacterial growth kinetics using the Varioskan LUX Multimode Microplate Reader

Ilkka Miettinen,[1] Malena Skogman,[1] Eva Tas,[2] Adyary Fallarero[2]

[1] Anti-infective Research (AIR), Pharmaceutical Design and Discovery group (PharmDD), Faculty of Pharmacy, University of Helsinki, Finland; [2] Thermo Fisher Scientific, Vantaa, Finland



Goal

This technical note aims to demonstrate the suitability of the Thermo Scientific™ Varioskan™ LUX Multimode Microplate Reader for following bacterial growth kinetics, and it highlights the simplicity of calculating kinetic parameters with the aid of Thermo Scientific™ SkanIt™ Software.

Introduction

A common basic step prior to starting a project involving bacteria is to study the growth of the selected model microorganism. This is usually accomplished by following changes in the optical density (OD) of the bacterial suspension at around 600 nm. The phases of bacterial culture growth (lag, log (logarithmic, also called exponential), stationary, and death) are well known. In particular, the log phase has been extensively studied since it represents the phase where the bacteria multiply in a linearly dependent fashion, at a constant rate.

Understanding where the exponential phase takes place is crucial in antimicrobial discovery studies, as it allows optimization of the conditions needed for minimum inhibitory concentration (MIC) experiments. More recently, the importance of the stationary phase has been highlighted by studies linking the metabolically inactive state to bacterial persistence and multidrug tolerance [1]. Thus, interest in studying bacterial growth kinetics continues to rise.

A typical misconception is that changes in OD at ~600 nm are a measure of bacterial absorbance. When a bacterial culture is exposed to light, the light is scattered and/or absorbed, and the changes in OD of a liquid bacterial culture are the combination of light scattering and light absorption (Figure 1). However, most bacterial cultures do not strongly absorb visible light and light scattering is more prominent, especially when the sizes of the particles (in this case, bacterial cells) are close to the visible wavelength of the light [2]. Therefore, instead of absorbance, changes in OD are mostly a measure of the light scattered by the bacterial suspension that cannot reach the detector and falsely manifests itself as absorbance.

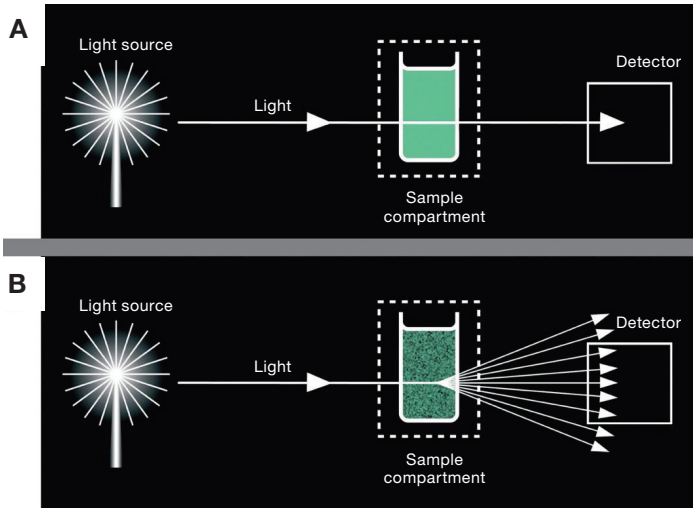


Figure 1. Difference between light absorbance and light scattering. **(A)** Attenuation of the light that reaches the detector is due to absorbance of light by the sample. **(B)** Attenuation of the light that reaches the detector is due to scattering of light by the sample. This decrease in the detected light creates the false result of an increase in the sample absorbance.

Following bacterial growth in a microplate reader requires that two parameters be adequately controlled: temperature and aeration. The optimal temperature for microorganisms to grow varies, but it is often around 37°C. For aeration, on the other hand, setting up the best parameters may be more challenging. Aeration is needed for bacterial growth, because oxygen typically does not dissolve well in liquids. Thus, unless oxygen can be supplied directly into the cultures, the best way to provide aeration is by shaking the microplate. Shaking can be accomplished in several ways, and the Varioskan LUX microplate reader offers orbital shaking. In this scenario, the diameter of the shaking pattern has a linear relationship to the rate at which oxygen is transferred from the air to the liquid, otherwise known as the oxygen transfer rate (OTR). Shaking in the Varioskan LUX microplate reader can be adjusted to be "Continuous" or "Pulsed", and the speed as well as shaking force can be selected, thus resulting in different orbital diameters. To avoid spillage of liquids, the SkanIt Software operating the Varioskan LUX reader will control the force and speed of the shaking based on the selected plate type. In addition, the total volume in the well should not exceed the maximum volume recommended by the plate manufacturer.

In this technical note, changes in the OD (at 595 nm) of *Staphylococcus aureus* and *Staphylococcus epidermidis* cultures were monitored using the Varioskan LUX reader during a 20 hr period in 96-well microplates. The overall reproducibility of the curves and the differences between growth kinetics of the two bacterial species were studied using SkanIt Software.

Materials and methods

Instruments

Varioskan LUX Multimode Microplate Reader

Bacterial strains, reagents, and materials

- *Staphylococcus aureus* (ATCC 25923)
- *Staphylococcus epidermidis* (ATCC 12228)
- Tryptone Soy Broth, TSB (Lab M, LAB004)
- Thermo Scientific™ Nunc™ MicroWell™ 96-Well Microplates, Nunclon™ Delta surface (Thermo Fisher Scientific, Cat. No. 161093)

Test setup

S. aureus and *S. epidermidis* cultures were started by inoculating 10 mL of sterile TSB with 30 µL of their respective cryopreserved stocks (pure cultures). Each suspension was mixed thoroughly and transferred to a clear 96-well microplate in volumes of 200 µL per well, 24 wells per culture. The bordering wells and the wells separating the two cultures were filled with 200 µL of sterile TSB. The plate was then inserted into the Varioskan LUX microplate reader with the lid on. A kinetic measurement of optical density (i.e., absorbance at 595 nm) was obtained every 15 min for a total of 20 hr, with incubation at 37°C and continuous low-force shaking at 60 rpm.

Results and discussion

For analysis of growth kinetics, the first step usually involves subtracting the contribution of the culture medium, using the “Blank subtraction” step in the “Protocol session tree” of SkanIt Software. This allows prompt recognition of the OD changes caused solely by bacterial growth. An initial assessment of reproducibility can be obtained by selecting all the measured wells and right-clicking “Show graph”. Following that, a “Kinetic reduction” step can be added for performing all the graphical analysis and kinetic calculations.

In this study, the curves corresponding to *S. aureus* or *S. epidermidis* were easily recognized by their distinctive kinetic patterns (Figure 2). The reproducibility of the kinetic growth curves is striking—variation between replicate wells was low for both species.

When the growth kinetics of two different species are visualized, as in this study, it may be useful in some instances to exclude the differences in optical density of the initial cultures (at time 0). This can be accomplished in SkanIt Software using the “Baseline subtraction” tool (Figure 3). This feature allows subtraction of a baseline that can be defined from a selected number of readings from the beginning or the end of a kinetic curve. In this case, the curves shown in Figure 2 can be remade by performing baseline subtraction with the first two reads.

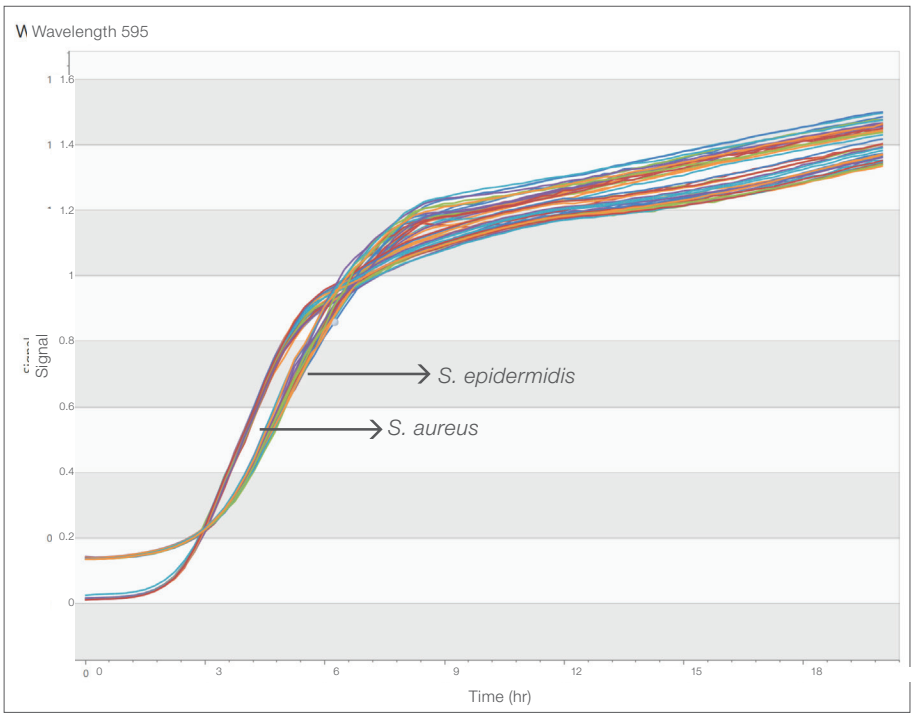


Figure 2. Kinetic curves obtained for *S. aureus* and *S. epidermidis* over a 20 hr growth period using the Varioskan LUX microplate reader.

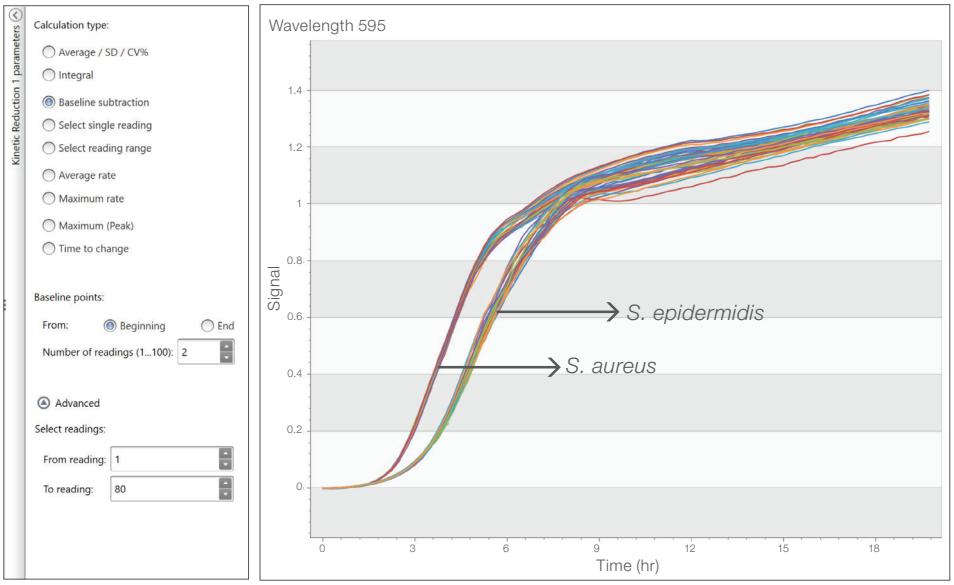


Figure 3. Example of the graphical processing that can be performed using baseline subtraction in SkanIt Software. The only difference from Figure 2 is the subtraction of the average of the first two reads, to improve the display of the curves.

It can also be concluded from Figure 2 that the exponential growth occurred at similar rates in both species, but the lag phase appeared to be shorter for *S. aureus* than for *S. epidermidis.* A quick way to quantify variation on the kinetic curves is to use the "Time to change" calculation provided by SkanIt Software (Figure 4A). With this parameter, the time needed to reach a certain absolute OD value in the curve or a certain percentage of OD change (positive or negative) can be calculated. For instance, if an OD value of 0.8, which is close to the upper absorbance limit of the exponential phase of both species, is selected, the software calculates that *S. aureus* reached the OD value of 0.8 in an average of 18220 seconds, or 5.06 hr (CV = 1.4%), compared to 20650 seconds, or 5.74 hr (CV = 1.9%) for *S. epidermidis*. These calculations are based on the originally obtained raw data of Figure 2.

For *S. aureus*, a closer inspection of the curves from Figure 2 also reveals that the exponential phase starts after read 10 (corresponding to 2.5 hr, since reads were performed every 15 min) and ends at around read 20 (corresponding to 5 hr). This analysis can be done by clicking on "Select reading range" and adjusting the reads in SkanIt Software. When this is selected, the graphs on the program will be automatically updated to this time frame (Figure 4B), and they can be further visualized on a single graph (Figure 4C).

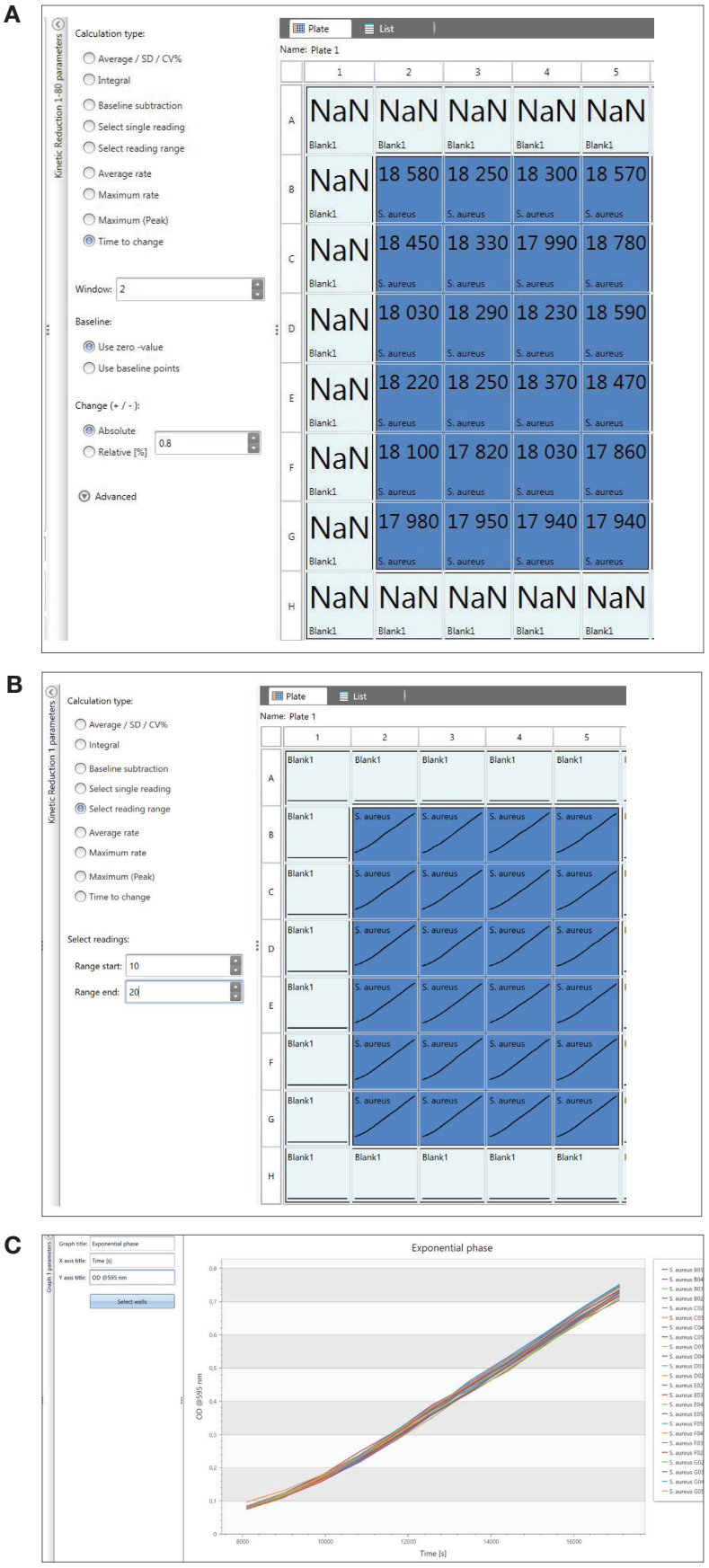


Figure 4. Examples of processing of kinetic data calculations, using SkanIt Software ("Time to change" and "Select reading range"). The results shown here correspond to the growth curve obtained for (24 replicate wells).

Once the exponential phase is defined to be between, for instance, reads 10 and 20 for *S. aureus*, the "Maximum rate" can be calculated (Figure 5). In this study, the maximum rate measured for *S. aureus* was 0.317 ΔOD/hr (average of the 24 replicate wells) with a calculated CV of 4.1%.

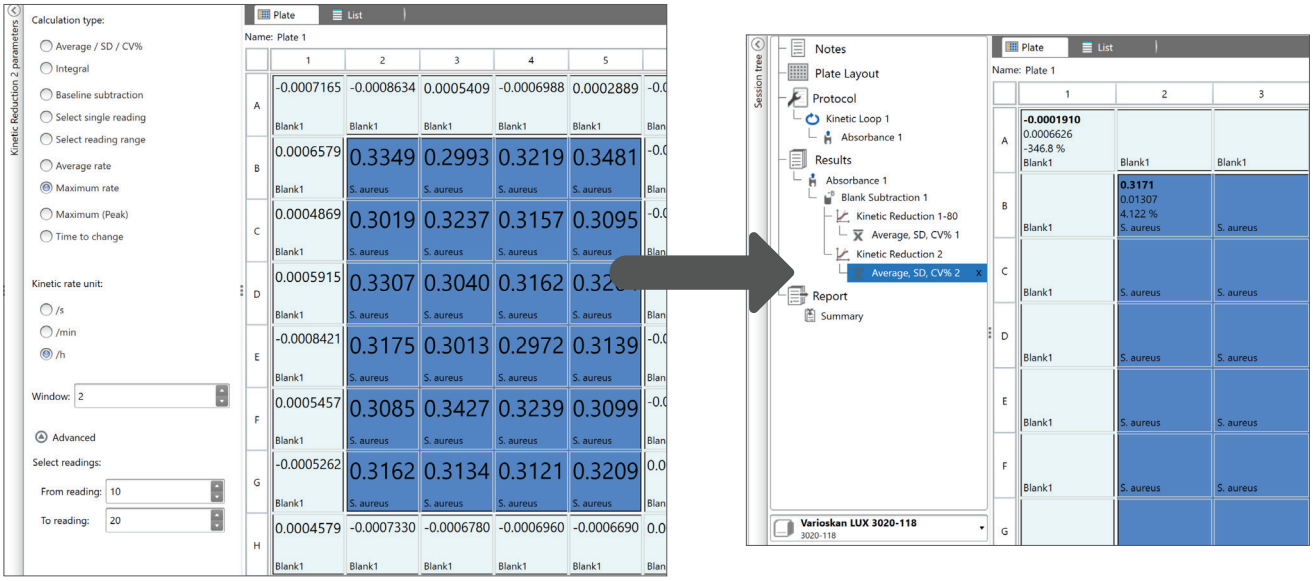


Figure 5. Illustration of the calculation of "Maximum rate" for the log phase of *S. aureus* (left). A step for calculation of "Average, SD, and CV%" of the maximum rate determined from replicate wells can also be added in SkanIt Software (right).

When the same type of analysis was carried out for *S. epidermidis*, the exponential phase was delayed and started only after read 12, lasting up to read 22. Performing a similar calculation of the maximum rate showed that *S. epidermidis* had a growth rate in the exponential phase of 0.290 ΔOD/hr (average of the 24 replicate wells) with a calculated CV of 11.4%. Shifting of the exponential phase by one read did not significantly change the maximum rate measured for the two species.

Further on, single-point measurements taken in the stationary phase (at 10 and 20 hr) were compared, to assess the variability between replicates. At 10 hr, the average of OD values for *S. aureus* was 1.138 with a calculated CV of 1.6%, while for *S. epidermidis* it was 1.208 with a calculated CV of 2.2% (24 replicate wells). At 20 hr, the average of OD values for *S. aureus* was 1.363 with a calculated CV of 1.7%, while for *S. epidermidis* it was 1.457 with a calculated CV of 1.5% (24 replicate wells). Thus, in the stationary phase, variability between replicates was also low, well under 5%.

Conclusions

Following bacterial growth kinetics is an indispensable routine in many laboratories. By using the Varioskan LUX Multimode Microplate Reader, these experiments become very straightforward. In particular, SkanIt Software provides a variety of kinetic calculations and data processing tools that ensure better, easier, and faster interpretation of microbiological results.

References

1. Conlon BP, Nakayasu ES, Fleck LE et al. (2013) Activated ClpP kills persisters and eradicates a chronic biofilm infection. 503(7476):365-370.

2. Lin HL, Lin CC, Lin YJ et al. (2010) Revisiting with a relative-density calibration approach the determination of growth rates of microorganisms by use of optical density data from liquid cultures. 76(5):1683-1685.

Find out more at **thermofisher.com/varioskanlux**