

Determination of Minimum Inhibitory Concentration (MIC) of antibiotics using OD₆₀₀

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Real-time analysis of microbial growth (OD₆₀₀) used for determination of MIC on BMG LABTECH readers

High-throughput capabilities and incubation options offer significant savings in time, efficiency and reliability

Subjective assessment of MIC eliminated through seamless, automated data acquisition and analysis

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INTRODUCTION

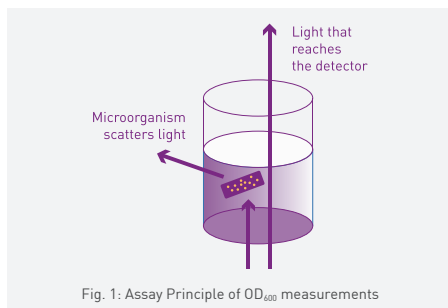
Today's escalating crisis of antibiotic resistance means that determination of the Minimum Inhibitory Concentration (MIC) is increasingly important to ensure effective antimicrobial therapy^{1,2}. MIC is defined as the lowest concentration of an antibiotic that inhibits microbial growth and is an essential parameter for dosing studies and research to contain the emergence of drug-resistant strains².

Microplate readers can be used for the determination of MIC using OD₆₀₀ measurements in low sample volumes^{3,4}. The use of microplates for the determination of MIC significantly increases throughput as multiple serial dilutions and replicates can be analysed concurrently. This high-throughput capability minimizes the variability of data and drastically reduces the time and labour associated with manual reading, ensuring a more robust and reproducible dataset. Moreover, automated data acquisition eliminates the subjective biases of conventional visual measurements of MIC and enhances accuracy.

In this application note, we demonstrate how a microplate reader can be used for the determination of MIC from OD₆₀₀ measurements.

ASSAY PRINCIPLE

The OD₆₀₀ measurement is a fast, non-destructive method to quantify the growth of microbes⁵. As bacteria grow, light scattering increases due to the increasing number of cells in the culture (Fig. 1). OD₆₀₀ measures light scattering through loss of transmission rather than absorbance per se which is typically non-existent. For the determination of MIC, OD₆₀₀ is used to distinguish between concentrations of an antibiotic that are sufficient to inhibit microbial growth and those that do not.

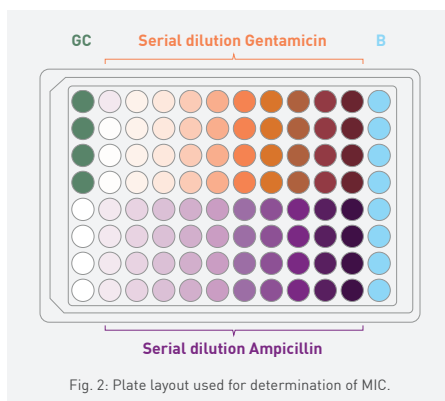


MATERIALS & METHODS

- 96-well plate, flat bottom, transparent, suspension plate (#650185, Greiner BioOne)
- Ampicillin (#HP62.1, Carl Roth)
- Gentamicin (#455310010, Thermo Fisher)
- LB broth (#10855021, Thermo Fisher)
- *Escherichia coli* (#6897, DSMZ)
- VANTastar® (BMG LABTECH)

Experimental Procedure

Cultures of *Escherichia coli* [*E. coli*] were thawed and inoculated for 18 h at 37 °C in 200 mL Luria-Bertani (LB) broth. 100 µL bacterial suspension was transferred into 50 mL fresh LB broth to prepare the *E. coli* stock. Gentamicin and ampicillin were dissolved separately in H₂O, filtered through a mesh with 0.22 µm for sterilisation and diluted in LB broth to prepare the antibiotic stock solutions. Two-fold dilutions of both antibiotics were prepared and 100 µL of each dilution was transferred into a 96-well plate (in quadruplicate). Next, 100 µL *E. coli* stock solution was transferred to each well. The final concentrations of the antibiotics were 0.1–50 µg/mL (Fig. 2). As a positive control for growth (GC), 100 µL *E. coli* suspension was mixed with 100 µL LB broth. The blank wells (B), which represent the assay background, included 200 µL LB broth only.



The accompanying lid with condensation rings was placed on the plate and OD₆₀₀ of the standards and samples were analysed for 12 h with the following settings in the VANTastar microplate reader for the determination of MIC.



Instrument settings

Optic settings	Absorbance, plate mode kinetic	
	Wavelength	600 nm
General settings	Number of flashes	22
	Settling time	0.1 s
Well Scan	Spiral averaging	2 mm diameter
Kinetic settings	Number of cycles	144
	Cycle time	300 s
	Shaking	Double orbital, 900rpm, shake between readings
Incubation	37 °C	

RESULTS & DISCUSSION

GC wells containing only *E. coli* without the addition of an antibiotic substance showed a substantial increase in OD₆₀₀ values over time. The culture showed a typical lag phase for growth. Samples treated with antibiotics showed a reduced increase in OD₆₀₀ over time, depending on the concentrations of antibiotics used (Fig. 3).

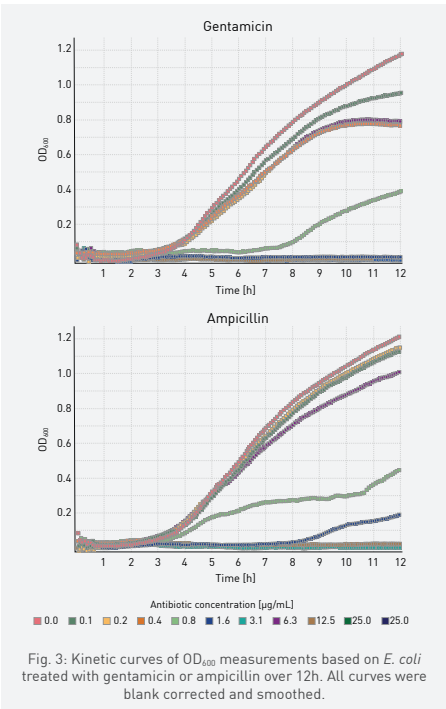


Fig. 3: Kinetic curves of OD₆₀₀ measurements based on *E. coli* treated with gentamicin or ampicillin over 12h. All curves were blank corrected and smoothed.

The OD₆₀₀ values after 12 h of incubation were compared to differentiate between preparations with and without bacterial growth. Therefore, all samples with an OD₆₀₀ value that is 3 standard deviations (SD) above the mean value of the blank wells were categorised as positive (Table 1) and used for the determination of MIC.

Table 1: Categorisation of *E. coli* samples with and without growth based on the OD₆₀₀ values after 12 h

Antibiotic concentration [µg/mL]	OD ₆₀₀ after 12 h-3xSD _{blank}	Validation	OD ₆₀₀ after 12 h-3xSD _{blank}	Validation
	Gentamicin		Ampicillin	
50.0	0.0	no growth	0.0	no growth
25.0	0.0	no growth	0.0	no growth
12.5	0.0	no growth	0.0	no growth
6.3	0.0	no growth	0.0	no growth
3.1*	0.0	no growth	0.0	no growth
1.6*	0.0	no growth	0.1	growth
0.8	0.3	growth	0.4	growth
0.4	0.8	growth	0.9	growth
0.2	0.8	growth	1.1	growth
0.1	0.9	growth	1.1	growth
*MIC Gentamicin		*MIC Ampicillin		

CONCLUSION

Measurement of OD₆₀₀ on a microplate reader provides a fast, reliable and objective method for the determination of the MIC value of an antibiotic substance. Microplate readers equipped with a UV-vis measurement option, allow the determination of bacterial growth in many samples in parallel, thereby providing a real-time, walk-away solution for the determination of MIC. BMG LABTECH readers permit microbes to be grown at a range of temperatures up to 65°C. The use of the optional Atmospheric Control Unit (ACU) also makes it possible to evaluate microbes, including fastidious organisms, with individual requirements for the concentration of O₂ and CO₂ gases in their culture environment.

The MARS Data Analysis Software simplifies the subsequent identification of samples with and without growth applying pre-defined templates. The underlying calculation streamlines the determination of MIC and eliminates subjective categorisation.

REFERENCES

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