



## Automated growth monitoring of microaerophilic organisms

Walkaway growth monitoring of *Helicobacter pylori* in the Infinite<sup>®</sup> 200 PRO reader with Gas Control Module (GCM<sup>™</sup>)

### Introduction

Growth analysis of microorganisms via OD absorbance measurements at 600 nm is key to many different research areas. In the past, this has been a time-consuming and labor-intensive procedure. Tecan's Infinite 200 PRO multimode reader can now provide all essential growth conditions – including continuous shaking, temperature control, O<sub>2</sub> and CO<sub>2</sub> regulation and humidity stabilization – within the measurement chamber, eliminating the need to transfer microplates between the cell incubator and the microplate reader (1). This enables simultaneous incubation and signal detection without the need for any manual intervention, even for microorganisms which need very specific environmental conditions, such as facultative anaerobic bacteria.

In this collaborative study, Tecan and its partners have analyzed the growth of the human pathogen and class-I carcinogen *Helicobacter pylori* over a period of 28 hours.

As a microaerophilic organism, *H. pylori* needs low atmospheric oxygen concentrations for optimized growth, and normally colonizes human mucosa, where it can cause certain types of stomach disorders and cancer. To mimic these physiological conditions, it is a prerequisite for the incubation/detection device to have the capability to control atmospheric O<sub>2</sub> levels.

Bacteria were incubated at 37 °C inside the measurement chamber of the Infinite 200 PRO reader, with continuous shaking, 10 % CO<sub>2</sub> and varying O<sub>2</sub> levels (normoxic (control), 5 % and 10 % O<sub>2</sub>). The proliferation was monitored by measuring sample absorbance at 600 nm, and the fluorescence of GFP-transformed *H. pylori*. To minimize evaporation effects during the long incubation period inside the reader, Tecan recommends using the Nunc Edge 96-well plate, which has been shown to minimize evaporation when used in combination the Infinite 200 PRO and the GCM (2, 3).

## Materials and Methods

- Infinite M200 PRO Quad4 Monochromators™-based multimode reader (Tecan, Austria)
- *H. pylori* (GFP-transformed P12, (2))
- Brain-heart infusion (BHI) medium (SIFIN GmbH, Germany) supplemented with 10 % horse serum (PAA, Austria), 1 % vitamin mix, 6 µg/ml chloramphenicol.
- Nunc™ Edge 96-well plate, transparent, cell culture treated (Thermo Fisher Scientific, USA) reader

GFP-expressing *H. pylori* were grown on agar plates containing 10 % horse serum under microaerophilic conditions at 37 °C for 48 hrs prior to harvesting.

Bacteria (107) in 200 µl culture medium were added to each well of a Nunc Edge 96-well plate. Evaporation of the culture medium was reduced by loading the plate moats with 1.5 ml of 0.1 % agarose.

Plates were covered with a standard microplate lid, transferred to the Infinite 200 PRO microplate reader, and the measurement protocol shown in Table 1 was started. Three different O<sub>2</sub> levels – 21 % (normoxia), 10 % and 5 % – were used to investigate the influence of the atmospheric O<sub>2</sub> concentration on the growth rate of *H. pylori*.

<b>Bacterial growth protocol</b>	
Plate	NUNC96ft_EdgePlate
Temperature	37 °C
Wait for temperature	36.5 - 37.5 °C
Kinetic cycles	29
Kinetic interval	30 min
Shaking	10 sec; amplitude: 3mm, mode: orbital
Wait	3 sec
Absorbance	600 nm Number of flashes: 25 Settle time: 50 ms
Shaking	10 sec; amplitude: 3mm, mode: orbital
Wait	3 sec
Fluorescence top	Excitation: 485 (9) nm Emission: 535 (20) nm Gain: optimal – use gain regulation Number of flashes: 20 Integration time: 20 µs Lag time: 0 µs Settle time: 0 ms Z-position: manual, 20,000 µm
Incubation 1	30 min; includes two shaking periods and zero waiting time: Shaking: 900 sec; amplitude: 3 mm; mode: orbital Shaking: 897 sec; amplitude: 3 mm; mode: orbital Remaining wait time
Incubation 2	25 min; two shaking periods and zero waiting time: Shaking; 900 sec; amplitude: 3 mm; mode: orbital Shaking: 597 sec; amplitude: 3 mm; mode: orbital Remaining wait time
GCM settings	CO <sub>2</sub> : 10 % O <sub>2</sub> : no regulation (21 %), 10 %, 5 %

Table 1 The *i-control*™-based protocol used to monitor growth of *H. pylori*

## Results and Discussion

As shown in Figures 1 and 2, accurate control of the O<sub>2</sub> level critically influences the proliferation of *H. pylori*. An optimal growth rate of *H. pylori* was found when 5 % O<sub>2</sub> was used inside the reader's measurement chamber. An exponential growth phase was identified between five and 22 hours incubation time, characterized by continuous data recording, even during the overnight period. Using the standard O<sub>2</sub> level of ~21 %, no growth could be detected at all, whereas 10 % O<sub>2</sub> results in a delayed growth of *H. pylori*, starting after approximately 20 hrs incubation time.

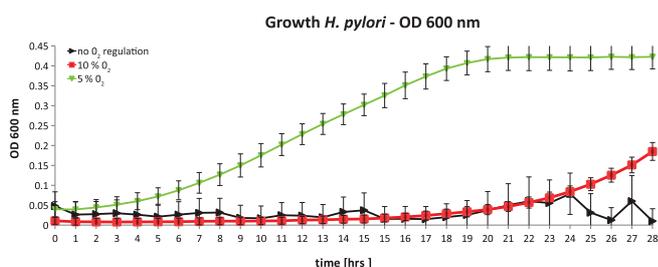


Figure 1 Growth of *H. pylori* monitored via 600 nm absorbance measurement

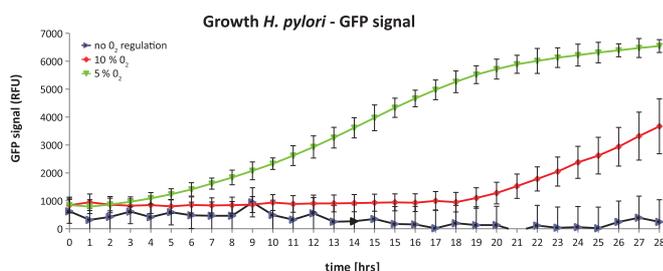


Figure 2 Growth of *H. pylori* monitored via GFP-fluorescence measurement

## Conclusion

This study clearly demonstrates the Infinite 200 PRO multimode reader's capability for automated growth monitoring of microaerophilic organisms such as *H. pylori*. The patent pending GCM technology enables the control of atmospheric O<sub>2</sub> and CO<sub>2</sub> levels inside the reader's measurement chamber which, combined with continuous shaking and heating, makes the Infinite 200 PRO the first hybrid incubator/reader on the market. Combining these incubator-related functions with the reader's multiple measurement modes allows fully-automated, continuous recording of real-time growth curves. Artefacts resulting from evaporation can be minimized by using the innovative Thermo Scientific Nunc Edge 96-well plate.

## Literature

- (1) [www.tecan.com/gcm](http://www.tecan.com/gcm)
- (2) Schmitt W, Haas R., Genetic analysis of the Helicobacter pylori vacuolating cytotoxin: structural similarities with the IgA protease type of exported protein. Mol Microbiol. 1994 Apr;12(2):307-19
- (3) Application Note. Analyzing biological processes. Long-term cell-based kinetics using Tecan's GCM and the Thermo Scientific Nunc Edge plate. Tecan Application Note 397050 V.1.0, 01-2012
- (4) White Paper. C. Oberdanner, A. Eger, J. Hinteramkogler, N. Jacobi, A. Koppensteiner, M. Pflüger, S. Verna, G. Probst. Inducing hypoxia inside Tecan's Infinite 200 PRO multimode reader. Tecan White Paper 397530 V1.0, 06-2012

## List of Abbreviations

GCM	Gas Control Module
GFP	Green fluorescent protein

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