Growth of *Neisseria meningitidis* in a BMG LABTECH microplate reader with Atmospheric Control Unit (ACU)

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- **Automated cell growth and culture studies using BMG LABTECH microplate readers equipped with ACU**
- ***Neisseria meningitidis* used to test Atmospheric Control Unit as it requires optimally 5% CO_2 to grow and thrive**
- **Improved growth rates observed in dilute cultures using the ACU as compared to using a CO_2 controlled incubator**

Introduction

It is well known that all organisms need a certain, but not necessarily a similar, level of carbon dioxide for growth and reproduction. The period during which this level is being increased corresponds to the lag phase as the organism is unable to divide until the critical concentration of CO_2 is reached.
The meningococcus has been considered one of the most fastidious micro-organisms with respect to growth requirements and it has long been recognized that most strains of *Neisseria meningitidis* require or benefit by, a concentration of CO_2 greater than atmospheric. Therefore, this organism is especially suitable for the study of CO_2 effect.
In this study, a strain of *Neisseria meningitidis* was used to assess the efficiency of a BMG LABTECH multimode plate reader coupled with an Atmospheric Control Unit (ACU) to deliver 5% CO_2. This was done by comparing growth of Neisseria meningitidis in the BMG LABTECH reader with a carbon dioxide incubator (set to deliver 5% CO_2) and in an incubator at atmospheric CO_2.

Materials & Methods

- BMG LABTECH microplate reader with ACU
- Lucy 1, Anthos, reader without atmospheric control

Neisseria meningitidis C751 was grown on Brain-Heart Infusion (BHI) agar supplemented with 10% foetal bovine serum (FBS) at 37°C and in 5% CO_2 overnight.
The following day, several colonies were resuspended in 10ml BHI broth supplemented with 10% FBS. This was serially diluted to 10^{-6} and 200 µl samples of each dilution were dispensed in triplicate into a sterile 96-well microplate. Absorbance readings were taken of each well every hour (ABS filter 405nm) in the BMG LABTECH plate reader with ACU (set at 37°C, 5% CO_2) over a 24h time period.

Identical microplates were placed in a 37°C, 5% CO_2 incubator and a 37°C incubator with no supplemental CO_2, and absorbance readings (at 405nm) were taken at 0, 1, 2, 3, 4, 6, 8 and 24h using an Anthos Lucy 1 microplate reader.
Once absorbance readings had been collected for all treatments, the blank media control (values not shown) was used to adjust values to represent increase in OD. Percentage bacterial growth was then calculated using the maximum OD achieved for each treatment as 100%.
Data was taken from triplicate wells on two separate occasions to give six data sets for analysis. Microsoft Excel was used to plot graphs and Minitab 13 was used to carry out tests of statistical significance.

Results & Discussion

From the data shown in Figures 1-3 it is evident that there is a clear correlation between starting inocula and growth rate for all three treatments.
At the higher starting inocula there appears to be little difference in bacterial growth between treatments. This is most likely due to there being a sufficiently high number of organisms present to produce a critical level of CO_2, therefore enabling initiation of growth without the need for an external CO_2 source.
However, differences in growth rate of *N.meningitidis* C751 as a result of CO_2 effect are more apparent in the most dilute cultures. For example, when grown at 37°C in atmospheric CO_2 (figure 1), the most dilute culture (1 in 1,000,000 dilution) exhibits a prolonged lag period due to the inability of the small inocula to reach the critical level of CO_2 to initiate growth. This lag phase is appreciably shorter when the same culture is grown in either a carbon dioxide incubator (figure 2) or the BMG LABTECH plate reader with ACU achieving 29% and 72% bacterial growth respectively after 24 hours incubation.
By comparing the data collected using a carbon dioxide incubator and the new BMG LABTECH's ACU (figure 2) it can be seen that there is less difference between growth rates of the serial dilutions using the BMG LABTECH plate reader. This is most likely due to a constant temperature and level of CO_2 being maintained throughout the duration of the experiment. Whereas, when using a CO_2 incubator the samples have to be transferred to a microplate reader to measure absorbance resulting in both CO_2 level and temperature fluctuations which ultimately result in decreased growth rate of *N.meningitidis*.

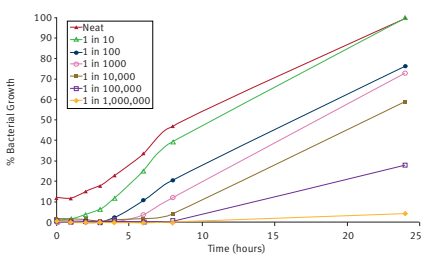

Fig. 1: Growth of serially diluted cultures of *Neisseria meningitidis* in BHI broth supplemented with 10% FBS at 37°C without supplemental CO_2. The data presented was calculated from triplicate optical density readings (at 405nm) taken in an Anthos Lucy 1 microplate reader from duplicate experiments.

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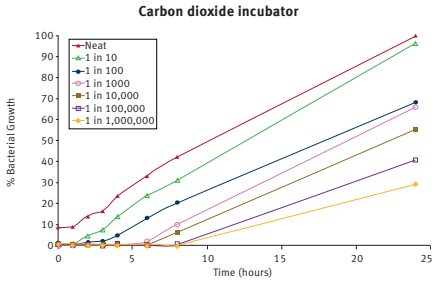

Fig. 2: Growth of serially diluted cultures of *Neisseria meningitidis* in BHI broth supplemented with 10% FBS in an incubator at 37°C, delivering 5% CO_2. The data presented was calculated from triplicate optical density readings (at 405 nm) taken in an Anthos Lucy 1 microplate reader from duplicate experiments.

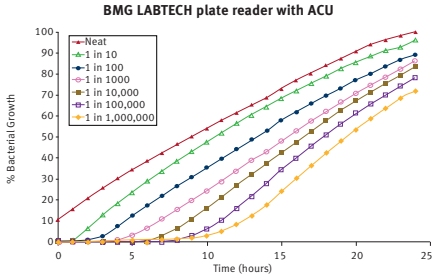

Fig. 3: Growth of serially diluted cultures of *Neisseria meningitidis* in BHI broth supplemented with 10% FBS using a BMG LABTECH plate reader with ACU set to deliver 5% CO_2 at 37°C. The data presented was calculated from triplicate optical density readings (at 405nm) taken hourly over a 24h period from duplicate experiments.

The results shown in Table 1 illustrate that this increased growth rate in the BMG LABTECH plate reader compared with a carbon dioxide incubator is statistically significant for all dilutions.

The results in Table 1 also reveal a significant difference in bacterial growth between the BMG LABTECH plate reader at atmospheric CO_2 for all except one dilution (1 in 10: p=0.052). However, the results also reveal that there is no significant difference in bacterial growth between a CO_2 incubator and atmospheric CO_2 which is most likely attributable to the temperature and CO_2 level fluctuations described earlier.

Table 1: Comparing growth of a serially diluted culture of *Neisseria meningitidis* C751 over a 24 hour period when grown at 37°C in a carbon dioxide incubator delivering 5%, in a BMG LABTECH plate reader with ACU delivering 5% CO_2 and with no supplemental CO_2 (atmospheric incubator). Minitab 13 was used to carry out t-tests and results were considered significant (+) or not significant (-) at the 95% confidence interval.

Comparison	CO_2 incubator vs BMG LABTECH	BMG LABTECH vs atmospheric incubator	CO_2 incubator vs atmospheric incubator
Dilution			
Neat	0.035 (+)	0.040 (+)	0.908 (-)
1 in 10	0.036 (+)	0.052 (-)	0.927 (-)
1 in 100	0.010 (+)	0.017 (+)	0.960 (-)
1 in 1,000	0.014 (+)	0.034 (+)	0.789 (-)
1 in 10,000	0.014 (+)	0.025 (+)	0.860 (-)
1 in 100,000	0.005 (+)	0.002 (+)	0.675 (-)
1 in 1,000,000	0.003 (+)	0.000 (+)	0.738 (-)

Conclusion

In conclusion, by utilizing the CO_2 dependency of a strain of *Neisseria meningitidis* it has been demonstrated that the BMG LABTECH plate reader with ACU is able to both achieve and sustain a level of CO_2 required for growth of such a fastidious organism. This study has also highlighted the advantage of this automated system over a carbon dioxide incubator. For example, conditions are more stable and the fact that it is less labour intensive means that a greater amount of data can be generated. This system is not only ideal for experiments using CO_2 dependent organisms such as *N.meningitidis* but may also prove very useful in cell culture studies.