Validation of MicrobesNG DNA shield buffer killing of *Coxiella burnetii* cultured in axenic media

Aim: To determine if DNA shield buffer supplied by MicrobesNG effectively kills *Coxiella burnetii* cultured in ACCM-2 media.

Method:

1. Inoculated 2 x T25 tissue culture flasks containing 10 ml ACCM-2 each with 1 ml *C. burnetii* NMI RSA 493 freezer stocks (≈1x10^6 CFU/ml).
2. Grew cultures for 7 days in an incubator set at 37 °C, 5% CO_2 and 2.5% O_2.
3. After 7 days, 9 ml of each culture was transferred to 15 ml falcon tubes and 100 µl of Fetal bovine serum (FBS) was added to aid pelleting.
4. Cells were pelleted by centrifugation at 5525 × g for 10 min (Sigma 4-16K benchtop centrifuge).
5. Supernatant was removed and cell pellets were resuspended in 500 µl PBS.
6. Cell suspensions were transferred to 1.5 ml Eppendorf tubes and cells were pelleted by centrifugation at 15100 × g for 5 min.
7. Repeat steps 5 and 6.
8. Cell pellets were inactivated by resuspension in 500 µl of MicrobesNG DNA shield buffer .
9. After 30 mins, 300 µl of inactivated cell suspension was pelleted by centrifugation at 15100g for 5 min.
10. Pellets were resuspended in 500 µl ACCM-2 media and used to inoculate (Day 0) 2 x T25 tissue culture flasks containing 10 ml ACCM-2 each.
11. Incubated flasks for 7 days in an incubator set at 37 °C, 5% CO_2 and 2.5% O_2.
12. On Day 0 and after 7 days incubation, 500 µl of culture was pelleted by centrifugation (15100 × g for 5 min) and DNA was extracted from cell pellets using Qiagen DNeasy® Blood and Tissue DNA extraction kit according to manufacturer protocol.
13. Extracted DNA was used to assess Coxiella growth after 7 days by qPCR targeting IS1111.

Control: At Day 0, 2 x T25 tissue culture flasks containing 10 ml ACCM-2 each were inoculated 300 µl of remaining culture in Step 3 above. Cultures were grown for 7 days, and DNA was extracted as above on Day 0 and Day 7 for analysis by qPCR.

Results:

- No growth was observed for cultures inoculated with *Coxiella* that was inactivated with DNA shield after 7-days (Figure 1).
- ≈ 10 – fold increase in growth was observed for untreated control cultures after 7-days.

Conclusions:

- MicrobesNG DNA shield lysis buffer inactivates ACCM-2 grown *Coxiella burnetii* after a minimum of 30 mins contact and following the procedure outlined above and in MicrobesNG guidelines (https://microbesng.com/documents/strain-submission/).

- Samples inactivated following this procedure can be sent to MicrobesNG for enhanced sequencing.
- (Optional): Cross check each inactivated sample to be sent for sequencing at MicrobesNG by culturing an aliquot in ACCM-2 to test for growth.

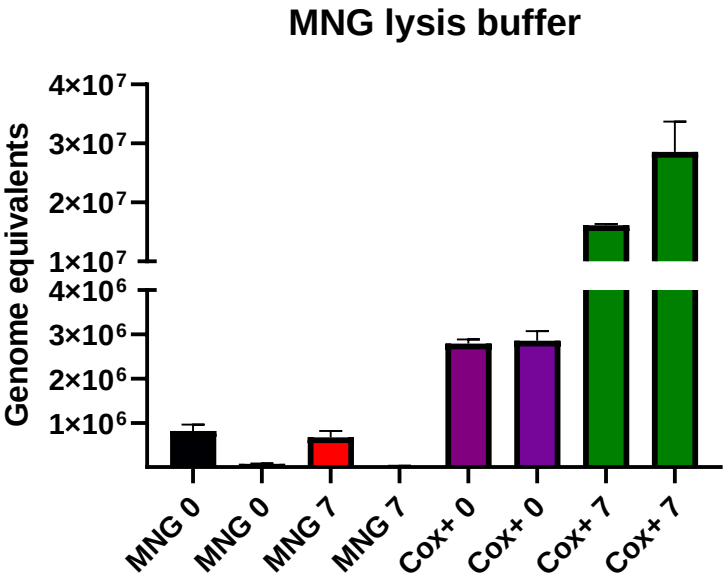

Fig 1: Quantification of *Coxiella burnetii* growth in ACCM-2 after inactivation with DNA shield.
C. burnetii genome equivalents were quantified by IS1111 qPCR from cultures treated with MicrobesNG DNA shield lysis buffer (MNG) and positive control samples (Cox+). Quantification was performed on samples taken on Day 0 and Day 7 for MNG (black and red bars) and Cox+ (purple and green bars). The mean and standard deviation for two qPCR technical replicates is shown for each sample.