Validation of MicrobesNG DNA shield buffer killing of *Coxiella burnetii* in mouse spleen homogenates

Aim: To determine if DNA shield buffer supplied by MicrobesNG effectively kills *Coxiella burnetii* present in homogenized mouse spleen tissue

Method:

1. Fresh mouse spleens were stored @ -80°C until start of procedure
2. Mouse spleens were allowed to thaw at room temperature.
3. Spleens were transferred to a MBSC and then transferred into a petri dish containing RPMI 1640 media.
4. 4 x spleens were chopped into small pieces using sterile dissection scissors
5. Each chopped up spleen was transferred to a single BD™ Medimachine tube and 1 ml RPMI 1640 was added to each tube.
6. Spleens were homogenized for 1 min in BD™ Medimachine and lysis mix was collected using a 2 ml syringe and transferred to sterile Eppendorf tubes.
7. 500 µl of ACCM-2 grown *C. burnetii* Nine Mile phase I (RSA 493) was added to each tube (n = 4) and left at RT for 30 mins
8. Tubes were centrifuged @1500 × *g* for 5 mins to remove eukaryotic cells and supernatant was transferred to sterile Eppendorf tubes.
9. Supernatants were centrifuged @15100 × g for 5 mins to pellet *C. burnetii* and supernatant was removed.
10. 3 x pellets were resuspended in 500 µl MicrobesNG lysis buffer and 1 x pellet was resuspended in 500 µl PBS (control). Tubes were left at RT for 30 mins.
11. 10 ml of 1X ACCM-2 in vented T25 flasks was inoculated with each sample and incubated @ 37°C, 5% CO_2 2.5 % O_2 for 10 days.
12. DNA was extracted from 200 µl of each flask prior to incubation (T=0)
13. After 10-days incubation, extracted DNA from 200 µl of each flask (T=10)
14. IS1111 qPCR was then performed on all samples to evaluate growth of *C. burnetii* in control and test cultures.

Results:

- No significant change in IS1111 Cq (quantification cycle) was observed for samples treated with MicrobesNG lysis buffer after 10-days (Figure 1).
- A significant decrease in IS1111 Cq was observed in untreated (control) cultures indicative of Coxiella growth in the absence of lysis buffer treatment (Figure 1).

Conclusions:

- MicrobesNG lysis buffer is effective at killing *C. burnetii* present in mouse spleen homogenates after a minimum of 30 mins contact time.

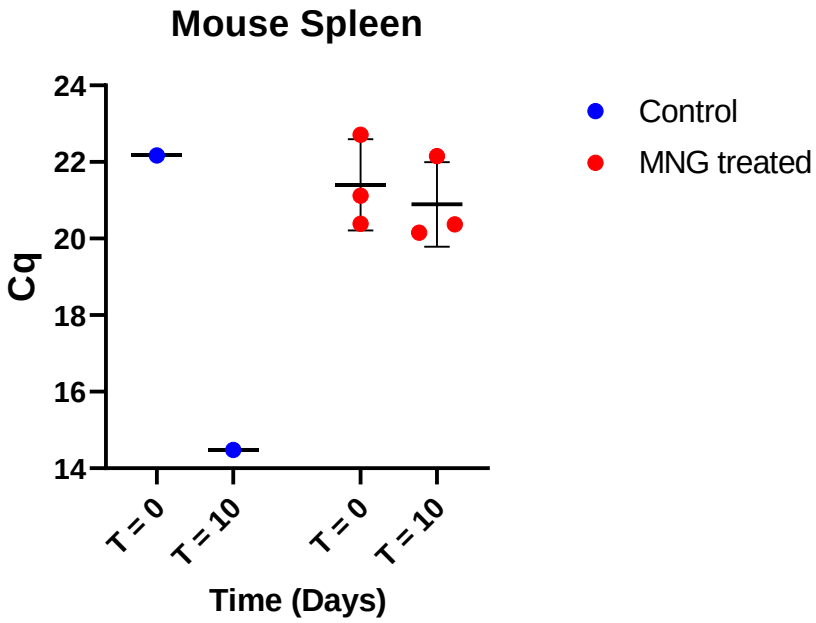

Fig 1: Quantification of *Coxiella burnetii* growth in ACCM-2 after inactivation with MicrobesNG DNA shield lysis buffer.

C. burnetii was quantified by IS1111 qPCR from ACCM-2 cultures of mouse spleen homogenates containing C. *burnetii* treated with MicrobesNG DNA shield lysis buffer (MNG, n=3) or treated with PBS as a positive control (Control, n=1). Quantification was performed on samples taken on Day 0 (T=0) and Day 10 (T=10). Horizontal bars represent the mean values.