Counting *Cryptosporidium*: Development of Simple *in vitro* Drug and Culture Platform

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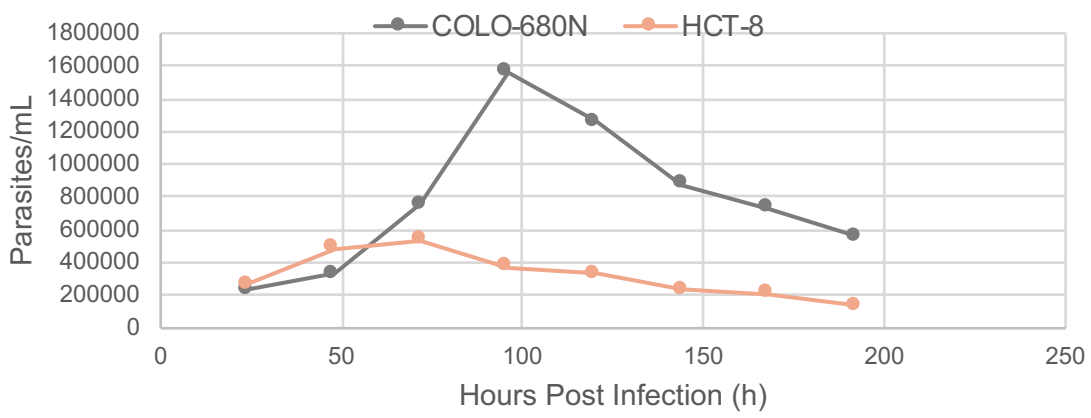
INTRODUCTION

Cryptosporidiosis is a disease which has a significant effect on immunosuppressed and young children. Caused by *Cryptosporidium spp*. this apicomplexan parasite can cause dehydration, malnutrition, muscle wasting and death in severe circumstances (1). We employ and optimise the *in vitro* cultivation method of *C. parvum* developed at the University of Kent using COLO-680N cells rather than the traditional HCT-8 cells (2). Optimisation of *in vitro* methods enables a cheaper and quicker methods to be developed as opposed to using animal models and animal vectors of proliferation. We down-scaled the culture platform from 5mL culture vessels, to 1mL cultures down to 200µL culture platforms. We hypothesise these platforms can be used to determine if a drug against *C. parvum* has an effect on parasite growth and proliferation *in vitro*. Using the 96-well plate, or 200µL, platform we have started to develop an Alamar Blue assay looking at host cell numbers after infection with *C. parvum*. Alamar blue is a metabolically activated dye, which living cells, such as those surviving infection with *C. parvum*, reduces resazurin to resorufin which is then detectable over time. We are aiming to optimise this method in order to develop a simple, quick and cost effective method of determining if various drugs have a protective effect on host cells challenged with *Cryptosporidium parvum*.

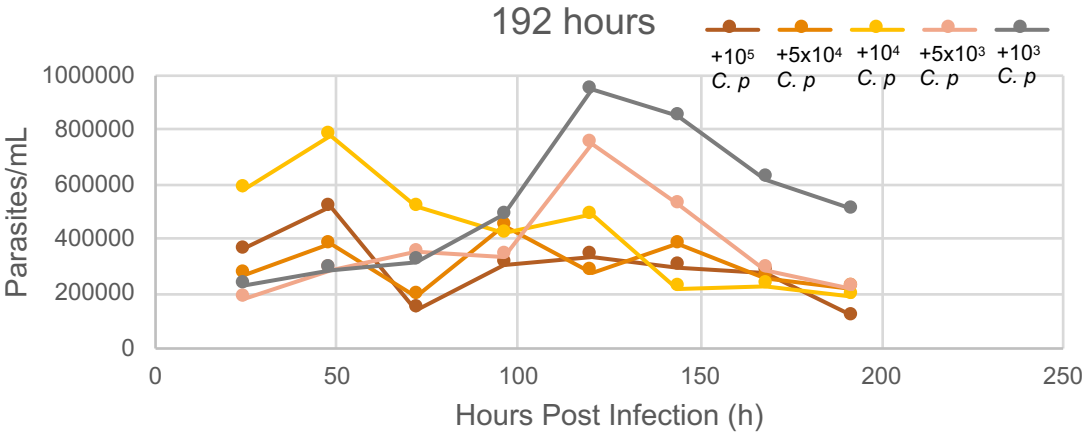
RESULTS

GROWTH IN COLO-680N SYSTEM:

A Average growth of *C. parvum* in **10⁶** COLO-680N versus **10⁶** HCT-8 Cells in a **5mL culture**



B Growth of a range of *C. parvum* concentrations in a **1mL culture** seeded with **10⁵** COLO-680N cells over 192 hours



C Growth of different concentrations of *C. parvum* in a **200µL culture** with wells seeded with **10⁴** COLO-680N cells over 192 hours

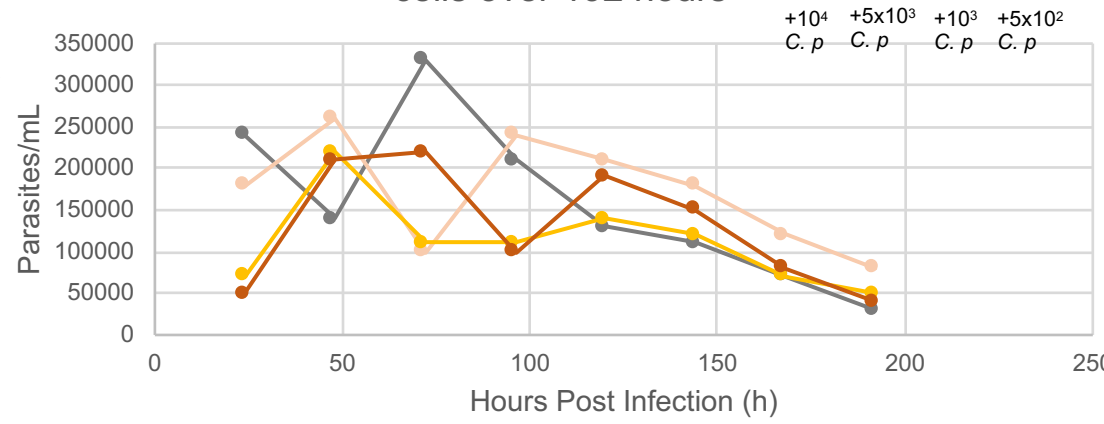


Figure 1 – Growth of *C. parvum* in the presence of COLO-680N cells or HCT-8 cells over the course of 192 hours (8 days). A) 5mL culture in T25 flasks. **B)** 1mL culture in a 24 well plate. **C)** 200µL culture in a 96 well plate. Cells were counted using a Neubauer Haemocytometer. Motile parasites were counted using a Neubauer haemocytometer every 24 hours for a total of 192 hours after infection with *C. parvum. C. p* stands for *C. parvum*.

- Results show each cell culture platform is viable for *C. parvum* growth and for subsequent drug testing experiments. Optimisation of each platform is currently underway.

INITIAL DRUG TESTING:

Growth of C. parvum in a **5mL culture** system with the presence of control drug Paromomycin

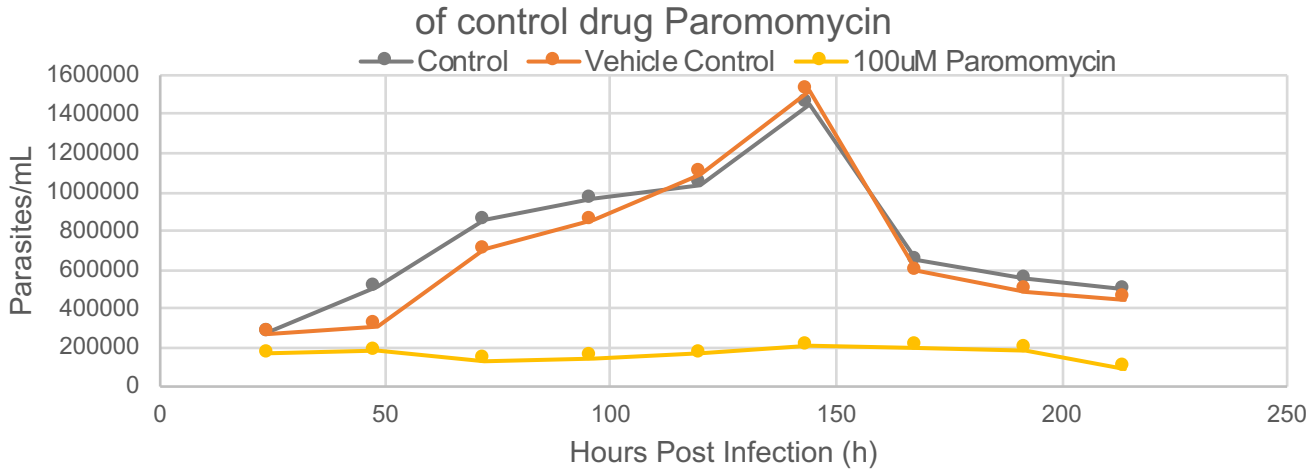


Figure 2 – Growth curves of *C. parvum* in COLO-680N cells in a 5mL culture system alongside 100µM Paromomycin. Flasks were seeded with 1×10⁶ cells/mL and were infected with 1×10⁶ sporozoites/mL 24 hours after seeding. 24 hours after infection with *C. parvum*, fresh media was placed into flasks. At this time media only was added to the 'Control' data, sterile distilled water was added as the 'Vehicle Control', and 100µM was added to the Paromomycin flask. Motile parasites were counted using a Neubauer haemocytometer every 24 hours for a total of 214 hours after infection with *C. parvum.*

- Paromomycin inhibits *C. parvum* proliferation in COLO-680N cells *in vitro*, and highlights the use of such culture platforms for drug hits against *Cryptosporidium parvum*.

ALAMAR BLUE ASSAY:

COLO-680N survival 6 days post infection with *C. parvum* measured using Alamar Blue over 5 hours (300 minutes)

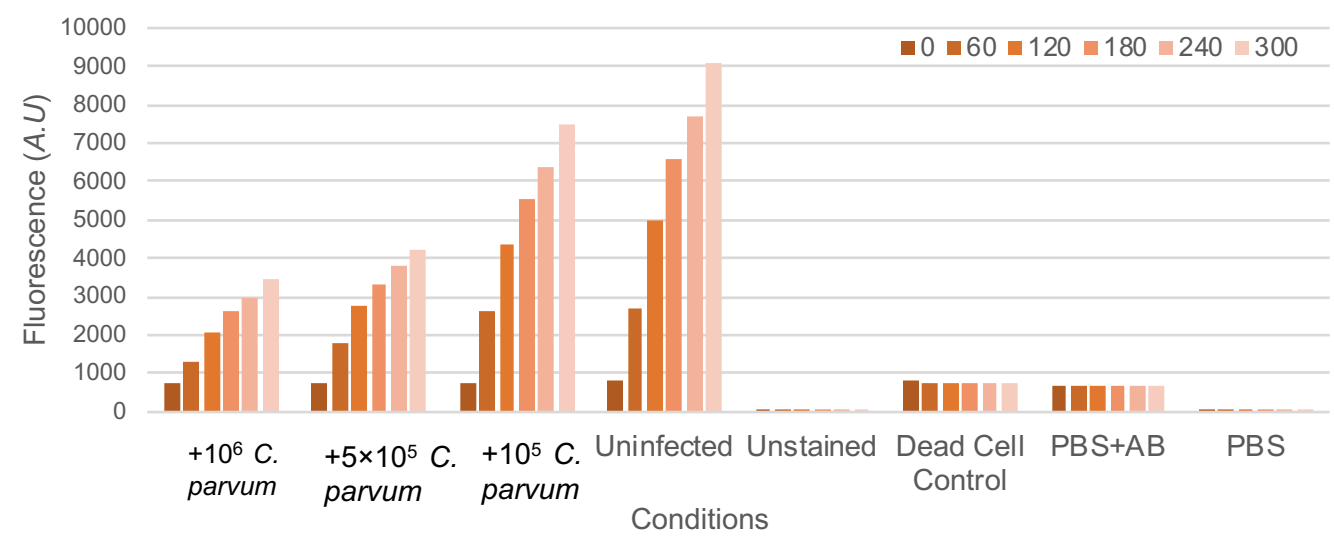


Figure 3 – Host Cell Protection Assay using Alamar Blue on COLO-680N cells 6 days post infection with *C. parvum*. A 96 well plate was seeded with COLO-680N cells at a concentration of 1x10³ cells/mL. 24 hours after seeding COLO-680N cells were infected with either: 1×10⁶, 5×10⁵ or 1×10⁵ parasites/mL. Controls were uninfected COLO-680N cells seeded at the same density as the test wells, unstained, dead cells which were exposure to 4% PFA, PBS only and PBS with Alamar Blue. 6 days post infection with *C. parvum* Alamar Blue was added and the fluorescence measured every 60 minutes for a total of 300 minutes.

- Alamar Blue can be used to measure the number of surviving host cells (COLO-680N) after infection with *C. parvum*. We are optimising this method to visualise drug protection of host cells from *C. parvum* related death *in vitro*.

CONCLUSIONS

- This platform can be used at various culture volumes and can be used to test for selective drug efficacy against *C. parvum*
- Alamar blue is not effective for merezoite detection from cell culture.
- Alamar blue shows promise when observing host cell survival when COLO-680N cells are challenged with *C. parvum* infection.

REFERENCES

1) Checkley W, White AC, Jr., Jaganath D, Arrowood MJ, Chalmers RM, Chen XM, et al. A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for cryptosporidium. Lancet Infect Dis. 2015;15(1):85-94.
2) Miller CN, Jossé L, Brown I, Blakeman B, Povey J, Yiangou L, et al. A cell culture platform for Cryptosporidium that enables long-term cultivation and new tools for the systematic investigation of its biology. Int J Parasitol. 2018;48(3-4):197-201.