Low-cost Cryptosporidium Pool Screen test to identify high-risk swimming pools

Seak Lin Ly, Water Quality Analyst, Sydney Water

Abstract:

In the summer of 2024, cryptosporidiosis outbreaks were linked to swimming pools in QLD, VIC, and NSW. However, community cases are under-reported. In Sydney Water's own wastewater monitoring results, there was a 100-fold increase in oocyst loads from winter 2023 to summer 2024.

The high cost of standard *Cryptosporidium* tests developed for drinking water make it economically challenging for routine swimming pool monitoring. Therefore, we are developing a cheaper test for screening swimming pools.

A range of swimming pools were sampled during the validation process. One litre of swimming pool sample was spiked with a known number of *Cryptosporidium* oocysts, filtered, then the captured oocysts were labelled and identified with fluorescence microscopy. Thus far, the median *Cryptosporidium* recovery is 92.5% (SEM± 4.3) (n=10) and the mode is 95%.

This method is highly specific for *Cryptosporidium*, but the small test volume means inherent low confidence results due to a high limit of detection (~1 oocyst/L). The intended purpose is, however, not to provide assurance that pools are free of *Cryptosporidium* but to provide a low-cost screening test to detect their presence at high concentrations. A limited quantitative microbiological risk assessment (QMRA) estimated that a single swimming session in a pool contaminated with 1 oocyst per litre would double a patron's daily risk compared to GI infections from all sources.

Introduction

Cryptosporidium is a protozoan parasite which, when ingested, causes the gastrointestinal disease cryptosporidiosis, characterised by water diarrhoea lasting several days to weeks. Historically many outbreaks of cryptosporidiosis are linked to swimming pools. (1–7). This is due to the tough outer wall of the oocyst which makes it highly resistant to chlorine. Oocysts can survive in swimming pool water for 10.6 days and can still be infectious to humans. Ingesting a dose as low as ten *Cryptosporidium* oocysts is enough to develop cryptosporidiosis (8).

In 2024, Australia had its worst outbreaks of cryptosporidiosis in 20 years. Over 13,000 cryptosporidiosis cases have been reported thus far (see Figure 1). This was four times more cases of cryptosporidiosis than previous years, with most cases presenting in young children less than 5 years old (see Figure 2).

However, cryptosporidiosis case numbers are underreported as many of those infected do not present for medical assessment. The diagnosis of cryptosporidiosis can only be determined by GP-prescribed pathology testing and it is through those pathology results that the national communicable disease surveillance dashboard gets updated (9,10).

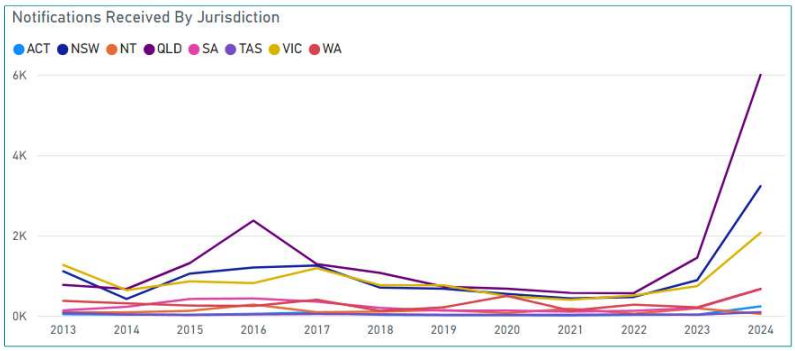

Figure 1. National Communicable Disease Surveillance program generated chart depicting reported cases of cryptosporidiosis in Australia from 2013 to 2024

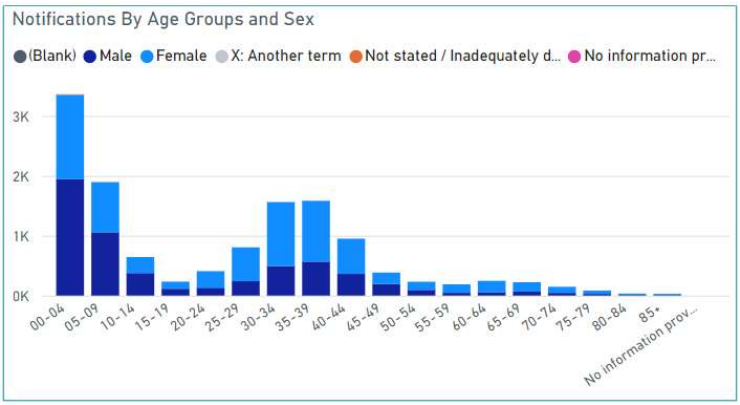

Figure 2. Distribution of 13,001 reported cryptosporidiosis cases amongst different age groups throughout Australia in 2024.

Wastewater testing provides another way to monitor *Cryptosporidium* circulating in the community. Testing during summer 2024 showed that in certain local government areas within the Sydney region, there was a 100-fold increase in *Cryptosporidium* concentrations in wastewater compared to the previous year.

Swimming pools are a common and well-recognised source of cryptosporidiosis outbreaks (1–7). The reasons for the persistence and reoccurrence of these outbreaks are multi-faceted, and include:

- High cost of and lack of access to specific water testing for *Cryptosporidium*
- Absence of bacterial indicators normally used in water quality monitoring of swimming pools not proving absence of *Cryptosporidium,* due to -
- High resistance of *Cryptosporidium* to inactivation by chlorine
- Lack of a visible trigger for super-chlorination.

An effective monitoring program may require frequent sampling (weekly). Few laboratories offer water testing for *Cryptosporidium,* and charges range from ~$500 to ~$1000 per sample, making routine swimming pool monitoring both economically and logistically challenging. The economic and practical barriers of water quality testing have led the Pool Water Testing Advisory Group (PWTAG) to advise against routine pool testing (8).

Super-chlorination levels of free chlorine are needed to effectively inactivate *Cryptosporidium.* Upon a positive *Cryptosporidium* detection or a faecal matter contamination event, the Australian public health guidelines require pool operators to super-chlorinate pools which means adding a high amount of chlorine to the swimming pool for a specific length of time (20mg/L for 13hrs, or 10mg/L of free chlorine for 26 hours) (11). This response may not even occur if there is a lack of visible faecal material when an infected patron contaminates the pool, but just 1mL of faecal matter contains ~1 million oocysts and an infected person may be unaware of the hazard they may cause as they continue to shed for 2 weeks after their symptoms subside (8). This, coupled with the long survival time in swimming pools and the small infectious dose, contributes to prolonged and heightened risk to swimming pool patrons. Pool operators cannot prophylactically super-chlorinate pools as a water quality management approach because the high use of chlorine is both costly and undesirable as it increases levels of harmful chlorine byproducts.

Results and Discussion:

Novel approach for a *Cryptosporidium* screening test

Collection and transport of standard 10L volume requested for *Cryptosporidium* analysis poses a logistical issue for routine testing, however, analysis of one litre of swimming pool water is adequate for a screening test that can identify if a swimming pool may pose a high risk to patrons. The PWTAG estimates that should a contaminated event occur where an infected patron releases faecal matter into a typical pool (25mx12m), then that pool would have an average concentration of 333 oocysts per litre (8).

A quantitative microbial risk assessment (QMRA) shows that if a pool has 1 oocyst per litre and assuming 50mL is ingested in a single visit, the disease burden per swimming event is 12 microDALY which doubles a patrons' daily risk for a GI infection:

Quantitative microbial risk assessment calculation

$$Disease\ burden\ = \left(\frac{oocysts}{L}\right) \times\ fraction\ oocysts\ human\ infectious\ \times$$
$$fraction\ oocysts\ viable\ \times\ volume\ (L)\ ingested\ per\ event\ \times$$
$$\#\ swimming\ sessions\ per\ day\ \times\ fraction\ of\ population\ susceptible\ \times$$
$$probability\ of\ infection\ per\ oocyst\ \times\ probability\ of\ illness\ per\ infection\ \times$$
$$DALY\ per\ case\ of\ cryptosporidiosis$$

Disease burden = N x 100% x 100% x V x 1 x 100% x 20% x 70% x 0.0017.*

*Values are assumptions taken from Health-Based Targets for drinking water. Average GI disease burden in Sydney is 2,300 microDALY per person per year (pppy), which equals 6.3 microDALY pp per day (12).

Therefore, given that 1 oocyst per litre is sufficient to double a patron's daily risk for GI infection and that a truly contaminated pool can have on the order of 300 oocysts per litre, then only a small sample volume is required to identify a high-risk swimming pool.

Screening test method development

Since March 2024, the entire method development process has involved trialling about 6 methodological variations. A wide range of pool matrices are being assessed as there is diversity in pool types, management, and usage levels – a total of 13 different pools and recreational bodies of water across 8 different sampling locations have been tested.

Thirty trials have been conducted to date, plus over 17 blanks to assess cross-over contamination. There have been no *Cryptosporidium* detections so far, possibly because the feasibility study was conducted mostly in winter when case numbers are low and there are fewer swimmers.

Since the method was established and protocols were finalised, a spike study has begun to measure recovery rates, as a reliable high recovery rate is necessary to guarantee that sensitivity of the test is sufficient. Samples were dosed with a standardised 100-oocyst spike. This also allows us to see whether swimming pool chemicals or debris will affect the appearance of *Cryptosporidium* oocysts and the analysts' ability to detect and identify them. The trial is in progress and so far, includes 10 pool samples processed (n= 10).

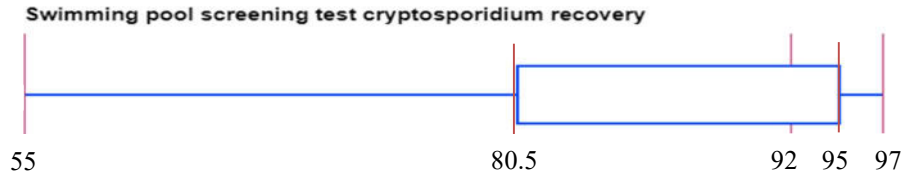

Figure 3. Box and whisker plot of pool screening test spike, recovery results

Table 1: Summary of *Cryptosporidium* recovery statistics and data distribution.

Number of spiked samples processed (n)	10
Mean (%)	86.2 ± 4.3 standard error
Median (%)	92.5
Mode (%)	95
Range (%)	55 - 97

The data dispersion is skewed, and all data points have been included from the method development process and initial set up. The outliers have been included and due to the small sample size and highly skewed data distribution the mean is not the most appropriate statistical parameter.

These trials also evaluate process speed and microscope slide readability. *Cryptosporidium* oocysts needs to be reliably distinguished and identified without background material obstruction or immunofluorescent antibody (IFA) cross-reaction, or stain retention issues.

Hyperchlorination experiment

A simulation of a pool hyperchlorination treatment was performed to see if the resulting damage to oocysts would render them undetectable. *Cryptosporidium hominis* was purified from stool samples of some anonymous patients that were infected with cryptosporidiosis (as confirmed by PCR) and composited. Artificial swimming pool water was dosed with liquid chlorine then spiked with ~5000 oocysts. The oocysts were exposed to 18mg/L free chlorine concentration levels over 1000min (equivalent to a super-chlorination event) and 50mL subsamples were taken at various time intervals (T= 0min, 100min, 1000min, 24hrs, 31hrs) to assess impact of exposure to high free chlorine levels and excessive contact time. The hyperchlorination experiment was conducted in pairwise duplicates. Quality controls included a non-spiked chlorinated test article for assessing free chlorine levels, and a non-chlorinated spiked test article as a staining control comparison.

The hyperchlorination experiment results showed that regardless of free chlorine concentration levels and exposure time, *Cryptosporidium* oocysts can be identified and possess a bright green, fluorescent signal. Even after 31 hours at free chlorine level greater than 20mg/L, 50% of the oocysts disintegrated, but the remaining 50% exhibited the bright green, fluorescent label.

Proposed sampling plan

The aim of the screening test is to assess whether the pool is a high risk to the public prior to use. We propose that sample collection should be carried out in the early morning prior to the swimming facility opening. This approach gives the swimming pool filter sufficient time to remove interfering debris like skin flakes and hair. This will improve test outcomes by minimising background material. Excessive amounts of skin flakes will negatively impact the test precision and the analyst's ability to distinguish and enumerate *Cryptosporidium* oocysts (see Figure 4).

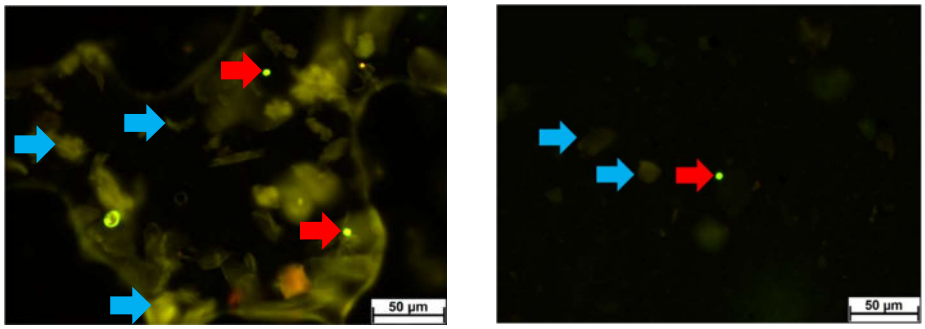

Figure 4. Left - heavy background interference, large amounts of skin flakes (blue arrows) are brightly stained, reducing microscope slide readability and increases reading time. Right - minimal background interference, some skin flakes (see blue arrows) appear faintly green. *Cryptosporidium* oocysts appear as bright green circles (see red arrows).

Standard commercial swimming pool filters are sufficient to deal with skin flakes but not microorganisms. The size of *Cryptosporidium* oocyst is between 4-6 µm, which can bypass most commercial pool filters which have a pore size of 600-800 µm (13).

Limitations of the study to date

The method development and validation study is not complete and requires further spike recovery testing from various pool matrixes throughout an entire year to assure robustness and precision of the test. After establishment, ongoing recovery quality control measures (1 in 20 samples, and for every new pool sample matrix), will enlarge the data base.

Due to the small volume tested, the method produces a low confidence result – a non-detect does not rule out the presence of *Cryptosporidium*. However, a positive result will identify high risk pools. More advanced methods can then be employed to confirm, quantify, and identify contaminating *Cryptosporidium* strains.

Conclusion

Reducing sample volume size and inventing a fast, inexpensive test that can identify high risk swimming pools will enable an economical approach to swimming pool monitoring for *Cryptosporidium.* The novel approach is still in development but is working well, and may enable monitoring regimes that permit better pool management strategies to help reduce cryptosporidiosis outbreaks in the future.

Acknowledgements

The author wishes to thank: Sydney Water Parasitology and Cell Molecular Biology team for their assistance during hyperchlorination experiment. Sydney Water Field Services team, Sample Receipt team, and Sydney Water Laboratory Services and Operations teams for operational support and logistics. The Commercial Customer Representative Supervisor Cheryl Novella for managing swimming pool and spa customers and organising sampling. The Microbiology Technical Specialist, Peter Beatson for his support and guidance during method development. Sydney Water Parasitology and Cell Molecular Biology Team Leader, Tanzin Ahmed and Biology Lab Manager Belinda Kinneally, for their support during the feasibility study. Catherine Cunningham and the Sydney Water Technical Services team for their expertise, and the time and resources they enabled through Sydney Water Innovations funding. The findings and conclusions in this report are those of the author and do not necessarily represent those of Sydney Water.

References

1. Joce RE, Bruce J, Kiely D, Noah ND, Dempster WB, Stalker R, et al. An outbreak of cryptosporidiosis associated with a swimming pool. Epidemiol Infect. 1991 Dec;107(3):497–508.
2. Mayne DJ, Ressler KA, Smith D, Hockey G, Botham SJ, Ferson MJ. A Community Outbreak of Cryptosporidiosis in Sydney Associated with a Public Swimming Facility: A Case-Control Study. Interdiscip Perspect Infect Dis. 2011;2011(1):341065.
3. Ryan U, Lawler S, Reid S. Limiting swimming pool outbreaks of cryptosporidiosis – the roles of regulations, staff, patrons and research. J Water Health. 2016 Nov 10;15(1):1–16.
4. Horne S, Sibal B, Sibal N, Green HK. *Cryptosporidium* outbreaks: identification, diagnosis, and management. Br J Gen Pract. 2017 Sep 1;67(662):425–6.
5. Lemmon JM, McAnulty JM, Bawden-Smith J. Outbreak of cryptosporidiosis linked to an indoor swimming pool. Med J Aust. 1996 Dec 2;165(11–12):613–6.
6. Puech MC, McAnulty JM, Lesjak M, Shaw N, Heron L, Watson JM. A Statewide Outbreak of Cryptosporidiosis in New South Wales Associated with Swimming at Public Pools. Epidemiol Infect. 2001;126(3):389–96.
7. Waldron LS, Ferrari BC, Cheung-Kwok-Sang C, Beggs PJ, Stephens N, Power ML. Molecular Epidemiology and Spatial Distribution of a Waterborne Cryptosporidiosis Outbreak in Australia. Appl Environ Microbiol. 2011 Nov;77(21):7766.
8. Pattinson M. What pool operators need to know about Cryptosporidium [Internet]. PWTAG - The Pool Water Treatment Advisory Group. [cited 2024 Oct 20]. Available from: https://www.pwtag.org/cryptosporidium-what-pool-operators-need-to-know-august-2015/
9. Ahmed SA, Karanis P. *Cryptosporidium* and Cryptosporidiosis: The Perspective from the Gulf Countries. Int J Environ Res Public Health. 2020 Sep 18;17(18):6824.
10. O'Leary JK, Sleator RD, Lucey B. *Cryptosporidium* spp. diagnosis and research in the 21st century. Food Waterborne Parasitol. 2021 Aug 20;24:e00131.
11. Cryptosporidiosis notification response plan; Diarrhoeal incident- public swimming pools and spa pools that use chlorine without cyanuric acid; Diarrhoeal incident – public swimming pools and spa pools that use chlorine with cyanuric acid. Available from: https://www.health.nsw.gov.au/environment/water/Pages/public-pools-and-spas.aspx [cited 20-10-2024].
12. Health Based Targets Manual. Water Services Association of Australia, October 2015. Available from: https://www.wsaa.asn.au/publication/health-based-targets-manual [cited 20-10-2024].
13. Wood M, Simmonds L, MacAdam J, Hassard F, Jarvis P, Chalmers RM. Role of filtration in managing the risk from *Cryptosporidium* in commercial swimming pools – a review. J Water Health. 2019 Mar 15;17(3):357–70.