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Cryptosporidium & Giardia in Water, Wastewater and Animal Scat

INTRODUCTION

Cryptosporidium and *Giardia* are protozoan parasites that infect and replicate within a host's intestine where they develop into the transmissible stages, oocysts and cysts. Infection is characterised by gastroenteritis and diarrhoea of varying severity and duration with oocysts and cysts being shed in large numbers in faeces. Shed oocysts and cysts are immediately infective but can also survive for several months in the environment.

ALS Water and Environmental laboratories provide **NATA accredited analysis of *Cryptosporidium* and *Giardia* in waters (ground, surface and potable), wastewaters, recycled water and animal scat in Victoria and NSW.**

SOURCES OF CRYPTOSPORIDIUM AND GIARDIA

Cryptosporidium oocysts and *Giardia* cysts can be introduced into raw water supplies either by direct or indirect contamination with human and animal waste and may be transmitted to humans through the ingestion of drinking water that has not been adequately treated. Waterborne outbreaks with either organism have occurred throughout the world. *Cryptosporidium* is one of the most important waterborne human pathogens given its resistance to common disinfectants (e.g., chlorine), size (ability to pass through treatment barriers) and low infectious dose. Catchments may be contaminated with human pathogenic *Cryptosporidium* oocysts shed in the waste of infected livestock and native animals.

Recreational waters (e.g. swimming pools) can also contain *Cryptosporidium* and *Giardia*. Infection outbreaks from swimming pools are usually more prevalent in the warmer months when pool use increases.

METHOD INFORMATION

ALS METHOD CODE:
MP546 (>500 mL), MP548 (<500 mL), MP550 (Animal Scat)
LIMIT OF REPORTING (LOR):
1 *Giardia* cyst and *Cryptosporidium* oocyst per volume/ amount analysed
METHOD REFERENCE:
USEPA 1623

GUIDELINES AND WATER INDUSTRY APPLICATIONS

The Australian Drinking Water Guidelines (ADWG) has no guideline value set for *Cryptosporidium* and *Giardia*; however, Cryptosporidiosis is a notifiable disease and most states require notification of Giardiasis. The relevant authority must also be notified if *Cryptosporidium* or *Giardia* is detected in drinking water. Monitoring of *Cryptosporidium* and *Giardia* can be used by the water industry to perform:

- Routine and event monitoring of drinking water.
- Validation and verification of water treatment processes.
- Quantitative microbial risk assessment (QMRA.)
- Catchment management surveys of animal scats.
- HACCP planning.

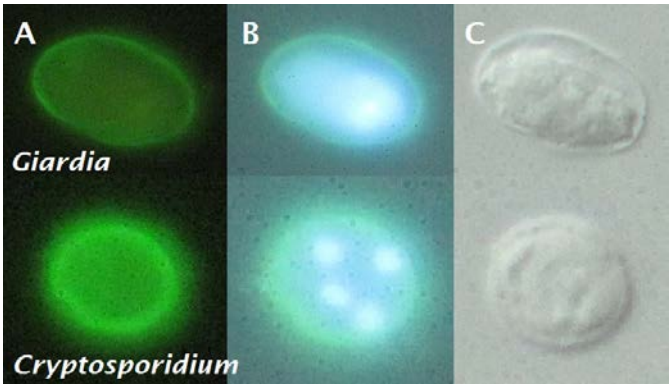

Figure 1: *Cryptosporidium* oocyst and *Giardia* cyst observed under (A) FITC fluorescent microscopy, B) DAPI fluorescent microscopy to identify nuclei, and (C) DIC microscopy to identify internal structures. Not to scale.

INDUSTRY PRACTICE AND TOOLS

Water Services Association of Australia (WSAA) recommend that water utilities with moderately or poorly protected catchments investigate the numbers of *Cryptosporidium* oocysts in their raw water to confirm that treatment processes are adequate and potentially obviate the need for capital expenditure. As raw wastewater contains oocysts at very high concentrations it is recommended that recycled water utilities conduct a QMRA to investigate, validate and verify treatment process performance based on the Australian Guidelines for Water Recycling (AGWR) requirements.

The ADWG recommends that water utilities investigate the numbers of *Cryptosporidium* oocysts and *Giardia* cysts in raw water supplies after an event that could increase the risk of contamination, e.g., the input of human and animal waste during heavy rainfall. Combining this information with animal scat catchment surveys is important to HACCP planning and event modelling. The NHMRC have indicated that a Health Based Target (HBT) will be used to manage pathogen risk in the next revision of the ADWG as part of the risk-based framework. A HBT defines a tolerable burden of disease. WSAA has developed an industry tool to calculate the burden of disease based on the number of *Cryptosporidium* oocysts detected. By calculating the burden of disease the tool also calculates the logarithmic reduction in oocysts required to achieve the HBT. An investigation of the number of *Cryptosporidium* oocysts in a catchment can therefore be used to assess the burden of disease and subsequently ensure that treatment process are adequate to achieve the HBT.

ANALYSIS OF CRYPTOSPORIDIUM AND GIARDIA

At ALS, samples are processed according to US EPA method 1623 for enumeration of *Cryptosporidium* and *Giardia* by microscopy. *Cryptosporidium* oocysts and *Giardia* cysts are concentrated from water and wastewater samples by filtration or direct centrifugation and separated from non-target debris by immunomagnetic separation. The final concentrate is applied to a microscope slide and stained with specific fluorescent dyes to identify the target organisms based on fluorescence, size and shape (Figure 1A) and the number of nuclei (Figure 1B). Differential interference contrast (DIC) microscopy (Figure 1C) is also used to identify the internal structures for confirmation.

Each sample is spiked with ColorSeed™ before processing. The Colorseed™ contains approximately 100 oocycts and cysts that fluoresce red under the microscope and so can be distinguished from any indigenous oocysts and cysts that may be present (Figure 2). The number of ColorSeed™ oocysts and cysts recovered is used to calculate the percentage recovery and to adjust the numbers of indigenous oocysts and cysts detected. The application of ColorSeed™ provides the highest level of quality control for any microbiological assay as it provides a measure of method performance for each and every sample.

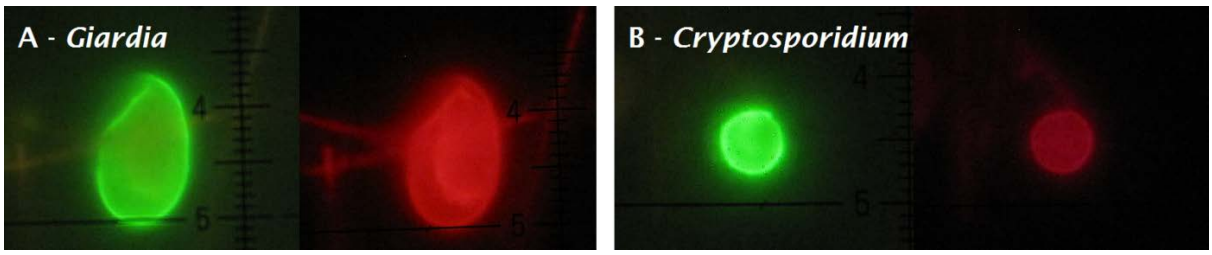

Figure 2: (A) Colorseed™ *Giardia* cyst observed under FITC (green) and red fluorescence, and (B) Colorseed™ *Cryptosporidium* oocyst observed under FITC (green) and red fluorescence. Not to scale.

The detection of *Cryptosporidium* oocysts by microscopy does not indicate whether the oocysts were alive or potentially human infectious. ALS have developed NATA accredited methods to detect and enumerate the number of infectious oocysts (EnviroMail 71) and determine whether the oocysts are human infectious genotypes (EnviroMail 66). This information can more accurately assess the risk to public health and whether treatment processes are appropriate.

SAMPLING REQUIREMENTS

Holding Time:	96 hours
Turnaround time:	<24 hours to 3 days (standard - from sample receipt at analysing laboratory
Sample Shipping and Storage:	≤30ºC
Sample Containers:	Clean plastic containers
Preservative	Sodium thiosulphate (for chlorinated supplies only)
Sample Volume:	Ground, Surface and Potable Water: 10–50 L (depending on turbidity of sample) Raw and treated wastewater: 1-20 L (depending on quality of wastewater) Recycled water: 50 L Animal Scat: 0.5 g

[1] *In hot conditions and/or remote locations requiring overnight air-freight, ALS recommends that containers be immediately refrigerated or placed in an esky upon sampling and covered with sufficient ice (or ice bricks) to chill the sample.*

For further information or to order sample containers please contact your local ALS client services team.