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Cryptosporidium Genotyping

INTRODUCTION

Following an extensive research and development project, ALS WRG (Melbourne) now offers NATA accredited Cryptosporidium genotyping from water and animal scat samples. The accreditation follows on from the successful completion of the WRF and USEPA sponsored project towards the development and validation of an international standard for the genotyping of Cryptosporidium.

WHAT IS GENOTYPING?

Genotyping is the process of determining differences in the genetic make-up of an individual organism by examining DNA sequences using biological assays and comparing it to a reference sequence.

Genotyping can be utilised to distinguish Cryptosporidium species or genotypes based on their genetic code.

WATER INDUSTRY APPLICATIONS

Not all species of Cryptosporidium are pathogenic to humans. By determining the species of Cryptosporidium present, the relative risk to human health can be assessed. This provides valuable information that aids in the:

- Development of effective watershed management plans;
- Validation and monitoring of treatment plants;
- Assessment of the public health risk from cryptosporidiosis.

WHO CAN BENEFIT?

Cryptosporidium genotyping is a valuable tool for any supplier of water destined for human consumption including Catchment Authorities, State Regulators, Water Supply Authorities, Recycling Scheme Operators and Local Councils.

METHOD INFORMATION

ALS METHOD CODE
W-CRYPTOG

LIMITS OF REPORTING (LOR)
1 oocyst per volume analysed

METHOD REFERENCE
Water Research Foundation Projects 4099 and 4284

WHAT IS CRYPTOSPORIDIOSIS?

Cryptosporidiosis is a diarrheal illness that affects immunocompromised and healthy humans, as well as agriculturally important livestock. Currently, there are 26 Cryptosporidium species and over 50 genotypes, however only a few (i.e. C. hominis and C. parvum) are infectious to healthy humans in all age groups. Therefore, in order to evaluate the human health risks of cryptosporidiosis, it is important to differentiate between Cryptosporidium species that cause the majority of human infections from those species or genotypes that primarily infect animals. This is particularly important for QMRA studies, HACCP planning and watershed management.

ANALYSIS OF CRYPTOSPORIDIUM

The standard method for detecting Cryptosporidium oocysts is microscopy based and cannot determine the species or genotype (See Figure 1). In order to determine species or genotype, any detected oocyst(s) are recovered from the microscope slide and subjected to PCR and DNA sequencing techniques to identify the genetic code. The PCR protocol is a single round of multiplex PCR targeting the Cryptosporidium genes for the 18S ribosomal RNA (18S rDNA) and the heat shock protein 70 (hsp70). If the 18S rDNA locus is detected, the sample is confirmed to contain Cryptosporidium. If the hsp70 locus is detected, the sample contains a human infectious species. Further DNA sequencing of the 18S rDNA and/or the hsp70 gene products will identify the species or genotype (See Figure 2).

Because oocysts are harvested for genotyping directly from the microscope slide this method can be used as an adjunct to the routine analysis of water samples for Giardia and/or Cryptosporidium.

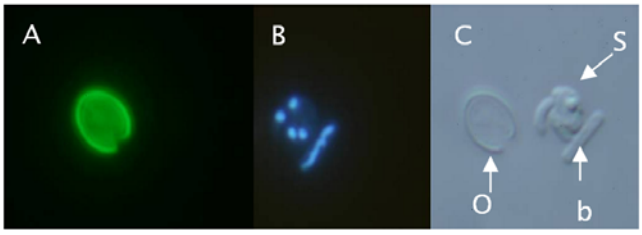

Figure 1: A) C. parvum oocyst observed under FITC fluorescent microscopy. B) The nuclei from each of 4 sporozoites observed under DAPI fluorescent microscopy. C) The sporozoites (S) and empty oocyst (O) observed under DIC microscopy. A bacterial cell is indicated (B)

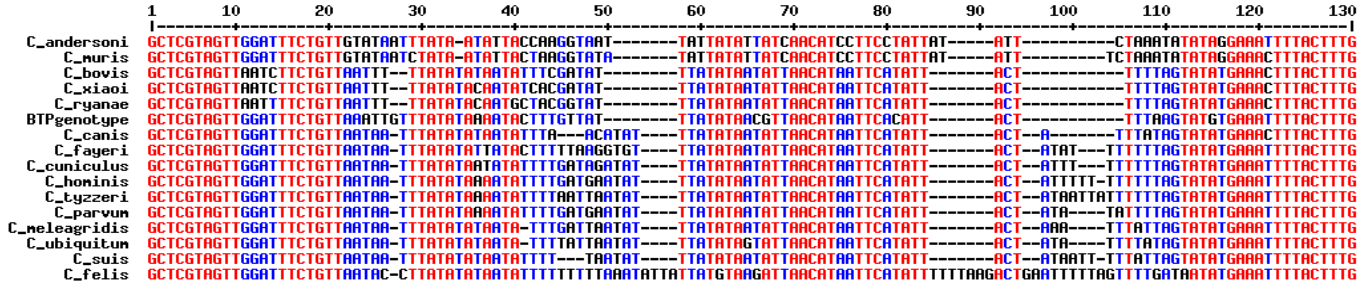

Figure 2: DNA sequence alignment of part of the 18s rRNA genes of different *Cryptosporidium* species. The nucleobases guanine (G), cytosine (C), adenine (A) and thymine (T) are indicated. Identical nucleobases are red, nucleobases that differ at a particular location are blue or black if they comprise the majority or minority consensus, respectively. Dashes indicate the truncation of the DNA sequence.

PROOF OF CONCEPT

Observed average detection rates from samples that contain only one oocyst and from samples that contain multiple oocysts (as identified by microscopy) were of 57% and 100%, respectively. Using the standard method for *Cryptosporidium* genotyping, the following species and genotypes have been identified by DNA sequencing from water and scat samples: *C. andersoni C. bovis, C. cuniculus, C. hominis, C. meleagridis, C. muris, C. parvum, C. ryanae, C. suis, C. xiaoi,* brush tail possum (BTP) genotype and the cervine genotype.

SAMPLING REQUIREMENTS

Holding Time:	4 days
TAT:	5 – 7 days (from sample receipt in Scoresby)
Sample Shipping and Storage:	<20ºC[1]
Sample Containers:	Clean plastic containers[2]
Sample Volume:	Surface/Environmental Water: 10 – 50L, pending turbidity Swimming pools and water parks: 100L Secondary waste water: 10-20L Recycled water: 50L Groundwater: 100L Scat: 0.5g
[1]In hot conditions and/or remote locations requiring overnight air-freight, ALS recommends that containers be immediately placed in an esky upon sampling and covered with sufficient ice (or ice bricks) to chill the sample. [2]Chlorine decreases oocyst recovery and therefore samples treated with chlorine (i.e. drinking water and swimming pools) need to be neutralized with 10% sodium thiosulphate.	

For further information please contact your local ALS Client Services Team.

REFERENCES

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