

# Cryptosporidiosis (*Cryptosporidium* genus)

## Laboratory case definition

The Public Health Laboratory Network (PHLN) has developed standard case definitions for the diagnosis of key diseases in Australia. This document contains the laboratory case definition for *cryptosporidium* genus.

**Authorisation:** PHLN

**Consensus date:** 28 March 2017

---

## 1 PHLN Summary Laboratory Definition

### 1.1 Condition:

Cryptosporidiosis

#### 1.1.1 Definitive Criteria

- a. Detection of typically stained oocysts, 4 to 6 m-m using Modified Kinyoun acid-fast stain or direct fluorescent antigen (DFA);
- b. Positive immunodiagnostic detection result in faeces; OR
- c. Positive polymerase chain reaction (PCR).

#### 1.1.2 Suggestive Criteria

Nil.

## 2 Introduction

*Cryptosporidium* is a coccidian parasite belonging to the family Cryptosporidiidae. Originally only one species, *Cryptosporidium parvum*, was recognized and subsequently divided into different genotypes which were host adapted e.g. to humans (genotype 1), cattle (genotype 2), and dogs.<sup>1</sup> Reviews of *Cryptosporidium* taxonomy over the past 20 years have led to many of the host-adapted genotypes acquiring species status. There are currently over 30 recognised species of *Cryptosporidium*, with over

20 of these recorded as responsible for human infections<sup>2</sup>. However, the vast majority of infections in humans are caused by *Cryptosporidium parvum* and *Cryptosporidium hominis*<sup>3</sup>. The clarification of *Cryptosporidium* taxonomy is useful for understanding the biology of *Cryptosporidium* spp., assessing the public health significance of *Cryptosporidium* spp. in animals and the environment, characterising transmission dynamics, and tracking infection and contamination sources.<sup>3</sup> Indeed, different species of *Cryptosporidium* and subtypes of *C. hominis* have been associated with differing clinical outcomes and potential for outbreaks.<sup>4</sup>

All life stages of the parasite are intracellular. At the time of excretion, the oocysts contain four infectious sporozoites. After ingestion and excystation by the host, the anterior end of each excysted sporozoite adheres to the luminal surface of an epithelial cell until microvilli surround it, making it intracellular but extracytoplasmic. Once established, it begins the infective process, producing new oocysts. Each oocyst measures 4 to 6 mm in diameter, and is shed as two distinct types. Approximately 20% of excreted oocysts are thin-walled (meronts), environmentally sensitive, and excyst endogenously, resulting in auto-infection of the host. The rest are thick-walled oocysts that are environmentally resistant, are shed in the faeces or sputum, and are immediately infectious to other hosts. *Cryptosporidium* can be transmitted person-to-person in settings such as day care centres and institutions, along with men who have sex with men (MSM). Infection is highly associated with travelling and exposure to farm animals. Outbreaks linked to contaminated swimming pools are not uncommon. There have been a number of notable waterborne outbreaks, most specifically the Milwaukee outbreak, which resulted in more than 400,000 cases after a breakdown in the water treatment system.<sup>5</sup> Foodborne transmission has also been demonstrated, which may be as a result of poor hygiene practices of infected food handlers, or from contamination of food products with animal waste or infected water.<sup>6,7</sup>

*Cryptosporidium* is of particular concern for four reasons:

- a. The oocyst is extremely resistant to disinfection and cannot be killed with routine water-disinfection procedures;
- b. Currently available anti-parasitic therapies have low efficacy and may only be of use in those with an intact immune system;
- c. The mortality from infection in severely immunocompromised patients can be as high as 50-60%; and
- d. Animal and human faecal wastes are associated with transmission of the disease to humans.

## 3 Tests

### 3.1 Direct Microscopy

*Cryptosporidium* cannot be cultured *in vitro* by methods that are practicable for use in a diagnostic laboratory.

It can be detected in clinical specimens using stained preparations.

#### 3.1.1 Suitable specimens

Generally fresh stools are submitted for laboratory analysis, but faecal specimens stored in 10% formalin, 5% formalin or SAF are preferred since the *Cryptosporidium* oocysts are immediately infective on passage. Frequently, one stool may not be sufficient to make a diagnosis, particularly if the patient is in the process of recovery.

### 3.1.2 Test details – stained preparations

*Cryptosporidium* oocysts, because of their small size (4 to 6µm) and similarity to yeasts, are easily missed in direct faecal specimen wet mounts. They are best visualised using modified Kinyoun Acid-Fast stain<sup>8</sup> or fluorescent monoclonal antibody (FA) staining reagents. Other techniques for the detection of acid-fast structures in stool specimens include the modified Zielh-Neelsen and the modified iron-haematoxylin methods. Typically, more oocysts will be seen in watery stools, whereas they become increasingly difficult to find in normal stools. The FA stains have a high specificity but are also more expensive to use.

### 3.1.3 Test sensitivity

Staining sensitivity is very dependent on the quality of the specimen. Formed stools contain larger numbers of artifacts, making interpretation more difficult, particularly when low numbers of oocysts are detected. Staining methods have been reported to have approximately 70% sensitivity in comparison to immunofluorescent antibody stains.<sup>9</sup> Direct FA test sensitivity is very high and approaches 100%.<sup>10,11</sup>

### 3.1.4 Test specificity

Some acid-fast stains also stain yeast cells to produce red-coloured oval cells of the same size range. The modified Kinyoun stain provides the most clear-cut staining result. The direct FA stain is 100% specific.

### 3.1.5 Predictive values

A negative diagnosis from a Modified Kinyoun-stained preparation does not preclude the presence of *Cryptosporidium*. At least three consecutive specimens may be required.

### 3.1.6 Suitable test acceptance criteria

Either:

- a. Modified Kinyoun (or other acid fast) stain: red staining oocysts showing internal sporozoites around the cell wall rim (crescent moon appearance) or brightly red coloured whole oocysts of the appropriate size range
- b. Fluorescent antibody stain: brightly fluorescent oocysts of the appropriate size range after direct FA under a fluorescent microscope.

### 3.1.7 Suitable internal controls

Positive control material should be included with each staining run to assess the reliability of the staining reagents.

### 3.1.8 Suitable test validation criteria

Typical oocyst morphology using modified Kinyoun (or other acid-fast stain) or direct FA compared to positive control.

### 3.1.9 Suitable external quality control (QC) program

Royal College of Pathologists of Australasia (RCPA).

### 3.1.10 Special considerations

None.

## 3.2 Antigen Detection

### 3.2.1 Test details

Direct antigen detection of oocysts in faecal specimens using commercially available kits is now the preferred screening method of many laboratories. Enzyme immunoassays using microtitre plates are the standard methodology, while simple, rapid, immunochromatographic immunoassays are now also widely used, with several using monoclonal antibodies to detect both *Giardia* and *Cryptosporidium* in a single step.

### 3.2.2 Suitable specimens

Fresh or frozen stool specimens, or stool specimens preserved in formalin or sodium acetate-acetic acid –formalin (SAF).

### 3.2.3 Test sensitivity

Enzyme immunoassay (EIA) kits are reported to have sensitivities ranging from 70-100%.<sup>12</sup> The sensitivity of immunochromatographic assays can range from 47.2% to 70.6% for all *Cryptosporidium* species, with improved performance for detection of the two major human pathogens *C. parvum* and *C. hominis* (50.1% to 86.7%). The overall sensitivity for detection of species other than these two is <35%.<sup>13</sup>

### 3.2.4 Test specificity

Immunochromatographic tests may give false positive results, especially with blood stained faeces. Reported specificities for EIAs, however, remain high, with values ranging from 98.1% to 100%.<sup>4</sup> The CDC case definition for cryptosporidiosis was modified in 2010 as a result of poor test specificity for immunochromatographic tests, and requires confirmation of positive results with another method.<sup>14</sup>

### 3.2.5 Predictive values

Negative predictive values approach 100% for all immunodiagnostic detection tests. Positive predictive values are dependent upon the population studied, but can be as low as 56% when using rapid assays.<sup>15</sup>

### 3.2.6 Suitable test acceptance criteria

A positive immunodiagnostic detection test result as defined by the kit manufacturer.

Positives from immunochromatographic tests should be confirmed by another technique.

### 3.2.7 Suitable internal controls

Kit internal controls

### 3.2.8 Suitable test validation criteria

### 3.2.9 Suitable external quality control (QC) program

None available for immunodiagnostic assays.

### 3.2.10 Special considerations

Some commercial kits will not accept specimens in formalin, while others will accept formalinised specimens but not those in PVA. Fresh faecal specimens routinely demonstrate the best sensitivity.<sup>16</sup>

## 3.3 Nucleic acid detection

### 3.3.1 Test details

Molecular diagnostics are becoming routine for the detection of pathogens in faeces, including protozoa. Real time PCR offers improved sensitivity and specificity as compared to routine methods, reduced labour, high throughput, and the opportunity for speciation when required. The majority of commercially available assays target the 18S rRNA gene, though assays targeting the *Cryptosporidium* oocyst wall protein (COWP), gp60, actin, beta-tubulin, LAXER sequence, and Hsp90 genes have been described.<sup>17</sup> Several commercial kits are available that may be coupled to automated extraction methods for optimised workflow.

### 3.3.2 Suitable specimens

Fresh faecal specimens will deliver the best results for PCR. In general, fixed specimens (e.g. SAF, PVA) will interfere with the enzymic activity necessary for the PCR reaction to work, and are therefore contraindicated for testing. *Cryptosporidium* may be detected by PCR in respiratory specimens; however, it is important to check whether the method used is validated for non-faecal specimens.

### 3.3.3 Test sensitivity

Estimations of sensitivity for molecular methods are problematic in the detection of faecal parasites, due to the insensitivity of the gold standard methods. In general, the sensitivity of molecular methods is very high, in the range of 93-100%, though reports will differ depending on the gene target and the extraction method.<sup>18</sup> Extraction techniques must be robust as the oocyst wall is difficult to disrupt using routine methods, which may lead to false negatives. Extraction methods that combine enzymatic, chemical, and mechanical lysis techniques have been demonstrated to perform superiorly to those that use chemical techniques alone.<sup>18</sup> In addition, discrepancies in the detection of DNA when comparing 18S rRNA and LAXER targets have been described, with the latter demonstrating a reduced sensitivity.<sup>17</sup> This can also be seen when species other than *C.parvum/C.hominis* are the culprit.<sup>19</sup> Finally, prolonged storage of specimens prior to testing may lead to false negatives due to lability of parasite DNA within the faecal medium.<sup>18</sup>

### 3.3.4 Test specificity

Specificity for molecular methods routinely approaches 100%.

### 3.3.5 Predictive values

Reported positive predictive and negative predictive values are 94.6% and 99.7% in comparison to DFA.<sup>20</sup>

### 3.3.6 Suitable test acceptance criteria

Institutional and commercial Ct cut-off values typically range from below 35-40, accompanied by an appropriate curve. Depending on institutional protocols, extraction controls (e.g. EHV) may be included with each run to confirm the absence of inhibition.

### 3.3.7 Suitable internal controls

Positive and negative (i.e. no template) controls should be included with each run. Positive controls may be supplied by the manufacturer or may be produced in-house.

### 3.3.8 Suitable test validation criteria

If extraction controls are used, these must be detected for the run to be considered valid, along with appropriate results for the positive and negative controls. The amplification curve should possess a typical sigmoidal shape with linearity achieved during the exponential phase of amplification.

### 3.3.9 Suitable external quality control (QC) program

A pilot program for molecular diagnostics on faeces is being commenced by the RCPA in 2017.

### 3.3.10 Special considerations

Species other than *C. parvum* or *C. hominis* are more likely to infect immunosuppressed patients. Given the varied ability of PCR targets to detect these species, it is important to be aware that negative PCR results in certain populations do not rule out infection with *Cryptosporidium* spp., despite their improved sensitivity as compared to traditional methods.

## 3.4 Serological tests

Serum IgG, IgA, and IgM produced in response to *Cryptosporidium* infection has been detected by EIA about 10 days post-infection.<sup>21</sup> Serum IgM titres drop quickly following establishment of infection, but IgG titres can remain elevated for several months. They may persist for longer in children from developing countries, presumably in response to continued exposure.<sup>21</sup> Dot blots and Western blots have been developed in some research laboratories, but commercial kits are not available. Serological tests are useful for epidemiological studies and outbreak investigations, but are rarely useful for diagnosis of infection in individuals.

## 4 Typing and Subtyping Methods

### 4.1 Genotyping

Speciation and genotyping is possible through interrogation of the gp60 gene.<sup>22</sup> However, this is not routinely performed in diagnostic laboratories or in epidemiological investigations.

## 5 Laboratory Nomenclature for National Data Dictionary

### 5.1 Organism name(s) list

- *Cryptosporidium parvum*/*Cryptosporidium hominis*\*
- *C. felis*
- *C. canis*
- *C. meleagridis*
- *C. muris*
- *C. andersoni*
- *C. bovis*
- *C. cuniculus*
- *C. fayeri*
- *C. ubiquitum*
- *C. viatorum*
- *C. scrofarum*
- *C. suis*
- *C. tyzzeri*
- *C. erinacei*

\* These two species cause approximately 90% of human infections.

### 5.2 Typing/subtyping nomenclature list(s)

Not applicable.

## 6 References

1. Caccio, S., Homan, W., Camilli, R., Traldi, G., Kortbeek, T., and Pozio, E. 2000. A microsatellite marker reveals heterogeneity within human and animal genotypes of *Cryptosporidium parvum*. *Parasitology*. 120(Part 3):237-244.
2. Taxonomy – *Cryptosporidium*. (Internet) (updated 2017 Feb 15; cited 2017 Mar 1). Available from: <http://www.uniprot.org/taxonomy/5806>
3. Ryan U, Fayer R, Xiao L. 2014. *Cryptosporidium* species in humans and animals: current understanding and research needs. *Parasitology*. 141(13):1667-1685.
4. Fletcher SM, Stark D, Harkness J, Ellis J. 2012. Enteric protozoa in the developed world: a public health perspective. *Clin Microbiol Rev* 25(3):420-449.
5. Garcia, L.S., and Bruckner, D.A. 1997 *Diagnostic Medical Parasitology*, 3rd. ed., p54-83. ASM Press, Washington, D.C.
6. Quiroz, E.S., Bern, C., MacArthur, J.R., Xiao, L.H., Fletcher, M., Arrowood, M.J., Shay, D.K., Levy, M.E., Glass, R.I. and Lal, A. 2000. An Outbreak of *Cryptosporidiosis* linked to a food handler. *J. Infect Dis*. 181(2):695-700.
7. McKerr C, Adak GK, Nichols G, Gorton R, Chalmers RM, Kafatos G, Cosford P, Charlett A, Reacher M, Pollock KG, Alexander CL, Morton S. 2015. *PLoS One* 10(5):e0125955.
8. Morgan UM, Pallant L, Dwyer BW, Forbes DA, Rich G, Thompson RCA. 1998. Comparison of PCR and Microscopy for Detection of *Cryptosporidium parvum* in Human Faecal Specimens: *Clinical Trial J. Clin. Microbiol.* 36:995-998.
9. Chalmers RM, Campbell BM, Crouch N, Charlett A, Davies AP. 2011. Comparison of diagnostic sensitivity and specificity of seven *Cryptosporidium* assays used in the UK. *J Med Microbiol* 60 (Part 11):1598-1604.
10. Garcia, L.S., Shum, A.C., and Bruckner, D.A. 1992. Evaluation of a new monoclonal antibody combination reagent for direct fluorescent detection of *Giardia* cysts and *Cryptosporidium* oocysts in human fecal specimens. *J. Clin. Microbiol.* 30:3255-3257.
11. Siddons, C.A., Chapman, P.A. and Rush, B.A. 1992. Evaluation of an enzyme immunoassay kit for detecting *Cryptosporidium* in faeces and environmental samples. *J. Clin. Microbiol.* 45:479-482.
12. Checkley W, White, AC Jr, Jaganath D, Arrowood M, Chalmers RM, Chen X-M, Fayer R, Griffiths JK, Guerrant RL, Hedstrom L, Huston CD, Kotloff KL, Kang G, Mead JR, Miller M, Petri WA Jr, Priest JW, Roos DS, Striepen B, Thompson RCA, Ward HD, Van Voorhis WA, Xiao L, Zhu G, Houpt ER. 2015. A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for *Cryptosporidium*. *Lancet Infect Dis* 15(1): 85-94.
13. Agnamey P, Sarfati C, Pinel C, Rabodonirina M, Kapel N, Dutoit E, Garnaud C, Diouf M, Garin J-F, Totet A, Derouin F. 2011. Evaluation of four commercial rapid immunochromatographic assays for detection of *Cryptosporidium* antigens in stool samples: a blind multicentre trial. *J Clin Microbiol* 49(4): 1605-1607.



14. Cryptosporidiosis (*Cryptosporidium* spp.) 2012 Case Definition. (Internet) (updated 2012; cited 2017 Mar 1). Available from: <https://www.cdc.gov/nndss/conditions/cryptosporidiosis/case-definition/2012/>
15. Robinson TJ, Cebelinski EA, Taylor C, Smith KE. 2010. Evaluation of the positive predictive value of rapid assays used by clinical laboratories in Minnesota for the diagnosis of cryptosporidiosis. *Clin Infect Dis* 50(8): e53-55.
16. Van den Bossche D, Cnops L, Verschueren J, Van Esbroeck M. 2015. Comparison of four rapid diagnostic tests, ELISA, microscopy and PCR for the detection of *Giardia lamblia*, *Cryptosporidium* spp. and *Entamoeba histolytica* in feces. *J Microbiol Methods* 110: 78-84.
17. Le Govic Y, Guyot K, Certad G, Deschildre A, Novo R, Mary C, Sendid B, Viscogliosi E, Favennec L, Dei-Cas E, Frealle E, Dutoit E. 2016. Assessment of microscopic and molecular tools for the diagnosis and follow-up of cryptosporidiosis in patients at risk. *Eur J Clin Microbiol Infect Dis* 35: 137-148.
18. Paulos S, Mateo M, de Lucio A, Hernandez-de Mingo M, Bailo B, Saugar JM, Cardona GA, Fuentes I, Mateo M, Carmena D. 2016. Evaluation of five commercial methods for the extraction and purification of DNA from human faecal samples for downstream molecular detection of the enteric protozoan parasites *Cryptosporidium* spp., *Giardia duodenalis*, and *Entamoeba* spp. *J Microbiol Methods* 127: 68-73.
19. Laude A, Valot S, Desoubreaux G, Argy N, Nourrisson C, Pomares C, Machouart M, Le Govic Y, Dalle F, Botterel F, Bourgeois N, Cateau E, Leterrier M, Le Pape P, Morio F. 2016. Is real-time PCR-based diagnosis similar in performance to routine parasitological examination for the identification of *Giardia intestinalis*, *Cryptosporidium parvum*/*Cryptosporidium hominis* and *Entamoeba histolytica* from stool samples? Evaluation of a new commercial multiplex PCR assay and literature review. *Clin Microbiol Infect* 22(2): 190.e1-190.e8.
20. Madison-Antenucci S, Relich RF, Doyle L, Espina N, Fuller D, Karchmer T, Laines A, Mortensen JE, Pancholi P, Veros W, Harrington SM. 2016. Multicenter evaluation of BD Max Enteric Parasite Real-Time PCR assay for detection of *Giardia duodenalis*, *Cryptosporidium hominis*, *Cryptosporidium parvum*, and *Entamoeba histolytica*. *J Clin Microbiol* 54(11): 2681-2688.
21. McDonald V. 2011. Cryptosporidiosis: host immune responses and the prospects for effective immunotherapies. *Expert Rev Anti Infect Ther* 9(11): 1077-1086.
22. Ebner J, Koehler AV, Robertson G, Bradbury RS, Jex AR, Haydon SR, Stevens MA, Norton R, Joachim A, Gasser RB. 2015. Genetic analysis of *Giardia* and *Cryptosporidium* from people in Northern Australia using PCR-based tools. *Infect Genet Evol* 36: 389-395.
23. Further references concerning assay characteristics for commercial kits may be found in the package inserts.