

Title: **Clostridium perfringens* enteritis* LABORATORY CASE DEFINITION (LCD)

The Public Health Laboratory Network have developed a standard case definition for the diagnosis of diseases which are notifiable in Australia. This page contains the laboratory case definition for *Clostridium perfringens* enteritis

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1 PHLN SUMMARY LABORATORY DEFINITION

1.1 **Condition:**

Clostridium perfringens enteritis

1.1.1 Definitive Criteria

1. Detection of $>10^6$ *Clostridium perfringens*/gram of faeces in a faecal spore count from patients with an epidemiologically consistent picture of gastroenteritis.
2. Detection of *Clostridium perfringens* enterotoxin and/or detection of the *cpe* gene from the stools of patients with an epidemiologically consistent picture of gastroenteritis.
3. Detection of $>10^6$ *Clostridium perfringens* per gram of food in epidemiologically implicated food samples.

1.1.2 Suggestive Criteria

1. A cluster of cases with diarrhoea and cramps associated with an epidemiologically consistent picture of gastroenteritis.

1.1.3 Special Considerations / Guide for Use

1. Faecal spores counts from geriatric patients must be interpreted with care as elevated levels of *Clostridium perfringens* are not uncommon in stools from this age group.
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2 INTRODUCTION

Clostridium perfringens (formerly *Cl. welchii*) is a gram positive, spore forming anaerobic bacillus, which is widely distributed in nature and can be found in soil and the gastrointestinal tract of vertebrates. It causes two main clinical syndromes: gas gangrene, acquired when wounds become contaminated with soil and faecal material, and enteritis, usually as a result of food poisoning, first reported by McClung in 1945. This case definition will be confined to enteric disease caused by *Cl. perfringens*.

Five types of *Cl. perfringens* (Types A, B, C, D and E) are recognised based on the secretion of alpha, beta, gamma, epsilon and iota toxins. All Types have been associated with enteric disease in animals but only Types A and C have been shown to cause enteritis in humans. Type C strains have been associated with necrotic enteritis including Pig-Bel in New Guinea, a more severe syndrome than the disease typically caused by Type A strains.

In Australia, Type A food poisoning syndrome predominates and is characterised by diarrhoea and strong stomach cramps of short duration (usually 24 hours)

Spores of *Cl. perfringens* survive cooking and germinate during slow cooling and unrefrigerated storage to form vegetative cells which quickly multiply (generation time of 7 minutes at 42°C) but do not produce toxin in food. Meat and poultry dishes that have been cooked hours in advance and slowly cooled provide favourable anaerobic conditions and are commonly implicated, but vegetarian curry dishes have also been responsible for outbreaks. Symptoms occur 8-22 hours (mean 15 hours) after ingestion of food containing a large number of vegetative *Cl. perfringens* cells. The bacteria multiply in the gut for a brief period then sporulate (produce spores) releasing enterotoxin (CPE) into the lumen of the gut at the same time. (Bates & Bodnaruk, 2003). This contrasts with food poisoning caused by *Staphylococcus aureus* enterotoxin and *Bacillus cereus* emetic toxin where the toxins are preformed in food and ingested, resulting in a rapid onset of enteric symptoms (2-6

hours). The food poisoning syndrome caused by *Cl. perfringens* is similar to that caused by the diarrhoeal syndrome of *Bacillus cereus*, where the toxin is not preformed and intoxication occurs following ingestion of contaminated food.

Outbreaks typically have a single point source with no secondary cases. They may be investigated by demonstrating the presence of large numbers of *Cl. perfringens* in the faeces of victims and the implicated food and confirmed by molecular typing of isolates. *Cl. perfringens* Type A enterotoxin (CPE) is not formed in food but may be detected in faeces using commercial antigen detection kits. The *cpe* gene is carried chromosomally on food poisoning strains of *Cl. perfringens* and confers heat resistance on the organism, making it an ideal agent for multiplication in foods that are kept warm for long periods of time. This is particularly a problem in foods that are stored for periods in Bain maries at warm temperatures.

In addition to the classical food poisoning syndrome, two other manifestations of *Cl. perfringens* enteritis are worthy of mention. The first of these is Antibiotic Associated Diarrhoea (AAD), typically seen in nursing home outbreaks. The typical pattern of these outbreaks is person to person transmission over a number of days, rather than as a point source outbreak. Whilst AAD is still mediated by the *cpe* gene, this is carried on a plasmid rather than chromosomally, which assists in diagnosis. More recently, a more severe necrotising colitis has been described in psychiatric patients with Type A food poisoning who are on anti-cholinergic drugs. (Bos et al.2005, MMWR report, 2010) This results in faecal impaction and retention of toxin, leading to necrosis of the colon and death.

Cl. perfringens is not targeted by the routine enteric microbiology workup in diagnostic laboratories due to a lack of simple and affordable detection methods. Usually only reference laboratories with access to specific food microbiological media have the necessary skills to investigate *Cl. perfringens* food poisoning outbreaks. A lack of routine laboratory-based surveillance means notifications of food poisonings attributed to *Cl. perfringens* are rare, although outbreaks caused by it are thought to be common and should be considered when investigating outbreaks with typical epidemiology. A study in 1992 found that enterotoxin could be detected in 6.8% of faecal specimens from sporadic and apparently non-food related diarrhoea (Brett, 1992).

3 TESTS

3.1 Culture of Human Faeces

Examination of faeces by culture includes enumeration of both vegetative cells and spores. Diagnosis is complicated by the natural occurrence of *Cl. perfringens* in normal faecal flora in variable numbers, both as vegetative cells and spores. This normal flora component may multiply during the food poisoning episode, approaching the numbers of the causative strain. Spores do not generally occur in numbers above 10^6 / g in healthy adult faeces but elevated levels may be seen in asymptomatic elderly individuals.

3.1.1 Suitable and Unsuitable Specimens

Faeces, with details of time collected post onset of symptoms is required to determine the significance of results (the toxin is rapidly eliminated from the gut as a result of the profuse diarrhoea and can therefore be very difficult to detect in older specimens (> 48 hours). Samples should be maintained at 4°C as freezing will produce a log 3 reduction in viable cells.

3.1.2 Test Details

Vegetative cell and spore counts:

Faeces are commonly cultured on tryptose sulphite cycloserine agar (TSC) at tenfold dilutions from 10^{-3} to 10^{-5} before (for vegetative cells) and after heat treatment at 80°C for 10 min (for spores). Plates are incubated anaerobically at 37°C. The 10^{-3} dilutions of heated and unheated aliquots are also plated on blood agar and incubated at 37°C aerobically and anaerobically to demonstrate the typical colony morphology of any *Cl. perfringens* present.

In addition, faeces 3 days old or less are also examined for the presence of heat resistant strains. An aliquot of faeces (1 mL) is placed into freshly boiled cooked meat medium (CMM) and boiled for 1 hour then incubated overnight at 37°C. The CMM is then subcultured to blood agar and incubated anaerobically at 37°C overnight.

3.1.3 Test Sensitivity

The major factor impacting on results is the timeliness of specimens receipt following onset. Significant counts and toxin/*cpe* gene detection are more likely to be recovered if specimens are collected soon after onset of symptoms.

3.1.4 Test Specificity

High counts of *Cl. perfringens*, derived from the normal flora, may be seen in faeces of people with diarrhoea caused by other enteric pathogens. Testing specimens from multiple cases involved in the outbreak will improve the positive predictive value. Multiple colonies of *Cl. perfringens* should be examined for *cpe* (enterotoxin gene) to distinguish enterotoxin producing isolates from normal flora components.

3.1.5 Predictive Values and relevant populations

Interpretation of results needs to be made carefully depending on the circumstances of the outbreak (see 3.1.6 below). Nursing home outbreaks may be attributed to either *Cl. perfringens* food poisoning or AAD. Culture for *Cl. perfringens* should only be requested when an appropriate epidemiological picture is present.

3.1.6 Suitable Test Acceptance Criteria

Growth of typical black colonies (H₂S production) surrounded by a zone of precipitation (lecithinase reaction). The most important confirmatory tests for isolates are Gram stain, lecithinase production, lactose fermentation, gelatine liquefaction, motility test and nitrate reduction.

Diagnostic levels of *Cl. perfringens* from faecal spore counts and in incriminated foods, supported by either detection of toxin in stools or detection of chromosomal carriage of the *cpe* gene in isolates from foods and stools, supported by an epidemiological history which is consistent with an outbreak caused by *Cl. perfringens*. For nursing home outbreaks with an AAD epidemiology, detection of the *cpe* gene on a plasmid should support this diagnosis.

3.1.7 Suitable Internal Controls

Control cultures of *Cl. perfringens* on all media used should be included when anaerobic jars are put up to demonstrate recovery of the target organism under suitable anaerobic conditions.

3.1.8 Suitable Original Test Validation Criteria

Nil

3.1.9 Suitable External (QAP) Programme(s)

There is currently no external QAP programme offering testing of this nature. Given the poor survival of *Cl. perfringens* in clinical samples, particularly in refrigerated or frozen specimens, development of such a QAP programme would be extremely difficult to produce.

3.1.10 Special Considerations

Nil

3.2 Culture of Food Samples

The Australian Standard Method for the isolation and enumeration of *Cl. perfringens* from food (AS 5013.16-2006) should be followed. The primary method involves a pour plate analysis using tryptose sulphite cycloserine agar (TCS) to enumerate viable cells. The test is run in duplicate and the results from both sets of plates averaged to obtain a final count. Multiple dilutions of a suspect food should be plated when investigating an outbreak.

A Most Probable Number (MPN) procedure in cooked meat medium with added neomycin may also be used (green book).

3.2.1 Suitable samples

The entire portion of food should be sampled or representative samples should be taken from different parts of large volumes of food because contamination may not be evenly distributed. *Cl. perfringens* cells rapidly lose viability when frozen or stored for long periods under refrigeration. Samples which cannot be examined immediately should be treated with buffered glycerine-salt solution prior to freezing (USFDA BAM).

3.2.2 Test Sensitivity

As detailed earlier, test sensitivity is determined by the age of the sample and its subsequent storage conditions.

3.2.3 Test specificity

Highly specific if large numbers ($> 10^5$) of *Cl. perfringens* enterotoxin positive isolates are recovered which share an epidemiological link to isolates from the faeces of complainants.

3.2.4 Predictive values

Positive predictive values are high if suitable quality-controlled media is used and cultures are incubated anaerobically.

3.2.5 Suitable Test Acceptance Criteria

Growth of typical black colonies (H₂S production) surrounded by a zone of precipitation (lecithinase reaction). The most important confirmatory tests for isolates are Gram stain, lecithinase production, lactose fermentation, gelatine liquefaction, motility test and nitrate reduction.

3.2.6 Suitable Internal Controls

A positive control culture should be incubated anaerobically with each anaerobic jar of plates to demonstrate that the media is capable of supporting growth of the target organism on the day of culture under the appropriate conditions.

3.2.7 Suitable Original Test Validation Criteria

There is an Australian Standard Method for the recovery and enumeration of *Cl. perfringens* in food samples (AS5013.16-2006). As the matrix of the food can have considerable bearing on the recovery of the target organism, it is important that suitable validation data are available to support the recovery of the organism in the particular food matrix.

3.2.8 Suitable External (QAP) Programme(s)

There are a number of Food Microbiology QAPs that offer samples contaminated with appropriate target organisms, but the programme run by IFM is particularly flexible in its design of appropriate matrices and target organisms, including *Cl. perfringens*.

3.2.9 Special Considerations

Typical Gram positive bacilli may be visualised in Gram stain smears of implicated food samples. Lecithinase negative *Cl. perfringens* have been associated with outbreaks (Pinegar et al 77).

3.3 Enterotoxin (Antigen) Detection

Assays are performed on faeces, not food samples, as the diarrhoea is caused by the enterotoxin when it is released into the gut by sporulating organisms, rather than being produced in food and then ingested. Enterotoxin may be detected in faeces by Vero cell assays, ELISA and reverse passive agglutination (RPLA) tests.

In Australia the Oxoid PET-RPLA was often used previously but it appears that this product is no longer available. Some laboratories still use Vero cells to detect enterotoxin activity. ELISA assays are not marketed in Australia. Further information about the performance characteristics of the three methods may be obtained from Berry et al 1988.

3.3.1 Specimens

Faeces only

3.3.2 Test Sensitivity

All three assays are highly sensitive in detecting enterotoxin if specimens from multiple patients are collected less than 2 days after onset of symptoms. The Vero cell assay is least sensitive and its sensitivity is further reduced if testing is delayed, because the assay relies on detection of the enterotoxin's biological activity which wanes on storage (Berry et al 1988).

3.3.3 Test Specificity

The ELISA is highly specific. The RPLA may give false positive results which are generally at the lower limit of detection of the test. The Vero cell assay is the least specific and reproducible but is adequate for investigating outbreaks where faecal specimens from multiple patients are collected soon after symptoms develop.

3.3.4 Predictive Values

ELISA: Positive and negative predictive values are high.

RPLA: High Negative predictive value, lower positive predictive value.

Vero cell assay: lowest negative and positive predictive values.

3.3.5 Suitable Internal Controls

ELISA: according to Bartholomew et al (1985).

RPLA: according to the manufacturer's instructions.

Vero cell assay: appropriate dilutions of enterotoxin preparation (Guigliano et al, 1983).

3.3.6 Suitable Original Test Validation Criteria

As per published literature.

3.3.7 Suitable External (QAP) Programme(s)

None available

3.3.8 Special Considerations

Specimens from multiple patients should be obtained as early as possible after symptoms appear.

3.4 **Nucleic Acid Tests**

Assays are performed on multiple isolates of confirmed *Cl. perfringens* recovered from the culture of food and faecal samples. The targets include the toxin genes (for molecular typing) and in particular the *cpe* enterotoxin gene. In addition, targets to determine whether the *cpe* gene is carried chromosomally or on a plasmid are included.

3.4.1 Suitable and Unsuitable specimens

Crude TE (Tris-EDTA) boils of isolates of *Cl. perfringens* that have been confirmed biochemically and that belong to a defined outbreak with an epidemiology consistent with an outbreak of *Cl. perfringens* food poisoning.

3.4.2 Test Sensitivity

Test sensitivity is high if appropriate controls are used and they provide expected reactions.

3.4.3 Test Specificity

The various targets are highly specific when used in conjunction with appropriate controls.

3.4.4 Predictive Values

Positive and negative predictive values are high if primers are designed appropriately.

3.4.5 Suitable Test Acceptance Criteria

In-house assays should be validated according to the NPAAC Requirements for the Validation of In-house In Vitro Diagnostic Devices (IVDs).

3.4.6 Suitable Internal Controls

Suitable controls include isolates that have been shown to carry the *cpe* gene chromosomally and via plasmids, as well as a range of isolates of different toxin types. The plasmid *cpe* can have two organisational arrangements; either an IS 1470-like or an IS 1151 sequence can be immediately downstream of the plasmid *cpe* gene.

3.4.7 Suitable Original Test Validation Criteria

Validation criteria are dependent on possession of a large databank of cultures of *Cl. perfringens* from food poisoning outbreaks, as well as other human and animal isolates. This collection should encompass all the different toxin types (A-E), the Beta2 toxin and the different variations of the *cpe* gene.

3.4.8 Suitable External QC Programme

None available

4 AGREED TYPING & SUBTYPING METHODS

A range of typing methodologies have been proposed to compare isolates from human cases with those from incriminated foods. These include Pulsed Field Gel Electrophoresis (PFGE) and Amplified Fragment Length Polymorphism (AFLP). However, with the development of Next Generation Sequencing and its availability in a number of reference laboratories, this would be considered to be the method of choice for any future investigations. This is particularly important in the light of the increasing push towards a forensic microbiology approach in the investigation of food poisoning outbreaks and the requirement to generate typing data that can stand up to legal scrutiny.

4.1 Laboratory Nomenclature for National Database Dictionary

4.1.1 Organism Name(s) List

SNOMED-CT Concepts	Code
<i>Clostridium perfringens</i> (organism)	8331005
Infection caused by <i>Clostridium perfringens</i> (disorder)	65154009
Food poisoning caused by <i>Clostridium perfringens</i> (disorder)	70014009
<i>Clostridium perfringens</i> enterotoxin (substance)	116557000
<i>Clostridium perfringens</i> genotype determination (procedure)	121894001
<i>Clostridium perfringens</i> enterotoxin assay (procedure)	121965009

5 REFERENCES

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