The extensive range of Oxoid products for the culture, detection and identification of *Clostridium difficile* enables laboratories to provide clinicians with the information they need to make swift diagnosis and isolation of infected patients, so containing possible outbreaks of this life-threatening hospital-acquired infection.
What is *Clostridium difficile*?

*Clostridium difficile* is a naturally occurring Gram-positive, anaerobic spore-forming bacillus. It is widely found in soil and animals’ intestines. Up to 50% of infants may be colonised in the first few months, although disease is rarely present at this age. It is seldom found in the flora of normal adults.\(^2\)

*Clostridium difficile*, in its growing state, cannot survive outside the gut, but the spores are able to remain viable for long periods of time in the environment. These spores are resistant to heating, drying and a wide range of chemicals, including many disinfectants.

How does *Clostridium difficile* induce disease?

*C. difficile* infection (CDI) occurs when the normal balance of bacterial intestinal flora is disturbed, for example by antibiotic therapy or surgery. Under these circumstances the organism is able to flourish and produce toxins.

Do all strains of *Clostridium difficile* induce disease?

Both toxin A (enterotoxin) and toxin B (cytotoxin) are major virulence factors. Not all strains of *Clostridium difficile* produce toxin and therefore not all induce illness.

What are the clinical signs of CDI?

Diarrhoea is present in almost all patients and other symptoms include:

- Fever
- Nausea/loss of appetite
- Abdominal pain/tenderness

The spectrum of the disease ranges from a self-limiting mild diarrhoea to the severe illness characteristic of pseudomembranous colitis.

Who is at risk of infection?

People in good health are usually not infected by *C. difficile*. The following groups are most at risk:

- Elderly patients (over 80% of cases occur in people over 65 years old\(^3\))
- People undergoing general surgery\(^4\)
- Patients requiring prolonged treatment with broad spectrum antibiotics
- People who are already ill, such as those with chronic renal disease\(^5\) and oncology patients\(^6,7\)

The organism has been associated with outbreaks in elderly care homes and hospitals.\(^8\)

How is the infection transmitted?

Infected patients excrete large amounts of bacteria and spores in their liquid faeces. These spores survive in the environment and are highly infectious. They can be spread on the hands of patients, healthcare staff or other people who have come into contact with the infected person and from contaminated surfaces and the environment.

**Voluntary surveillance of *Clostridium difficile* in England, Wales, and Northern Ireland, 2008**

In 2008, there were 37,134 reports of CDI, comprising 32,602 from England, 2,860 from Wales and 1,672 from N. Ireland, reported to the HPA. There was a 35.1% decrease in the number of *C. difficile* laboratory reports compared to 2007.

The incidence rate of *C. difficile* per population has decreased in England and Wales from 104 to 63 and 97 to 96 samples per 100,000 population respectively, and increased in Northern Ireland from 79 to 94 samples per 100,000 population.

Around 80% of all laboratory reports for CDI were in people aged 65 years and over.

The number of labs across England, Wales and Northern Ireland reporting cases of *C. difficile* has decreased by 7% from 186 in 2007 to 173 in 2008.

Mandatory reporting of *C. difficile* in people aged 65 years and over was introduced in England in 2004, but has not replaced voluntary reporting.

There has been a decrease in the number of laboratory reports across England, Wales and N. Ireland, but the public health impact of *C. difficile* remains important. The downward trend suggests progress is being made in reducing infection, however, ongoing surveillance of this disease is vital.
How has the incidence of *C. difficile* disease evolved?

In the UK, the number of *C. difficile* isolates reported from faecal specimens increased by almost 200% from 22,008 in 2001 to 43,682 in 2004. An increase in the reporting of CDI rather than an increase in the prevalence of CDI could explain this alarming increase, but the true prevalence of *C. difficile* is likely to be very much higher than the number reported because these data are from a voluntary reporting scheme. A mandatory reporting scheme was introduced in January 2004 for *C. difficile* cases in patients 65 years or older (www.hpa.org.uk).

US hospital discharges for which CDI was listed as any diagnosis doubled from 82,000 or 31/100,000 population in 1996 to 178,000 or 61/100,000 in 2002. The overall rate during this period was several-fold higher in persons >65 years of age (228/100,000) than in the age group with the next highest rate, 45-64 years (40/100,000). CDI appears to be increasing rapidly in the United States and is disproportionately affecting older people.

There exists a severe underestimation of CDI in Europe due to a lack of awareness among physicians and lack of standardized diagnostic strategies. The association between CDI and antibiotic use is overestimated because the disease also occurs in patients without previous antibiotic treatment.

What are the criteria for CDI testing?

The main criterion for requesting laboratory diagnosis is symptomatic disease and, in most cases, CDI investigations are undertaken at the request of the physician. Patients suffering diarrhoea and/or abdominal pain should be tested when:

- The patient belongs to a high risk group i.e. over 65 years old, immunocompromised, severe underlying disease
- There is diarrhoea lasting more than 3 days without another known pathogen
- The patient has taken antibiotics in the past 30 days.

What are the different toxin detection techniques?

1. Cytotoxicity activity (CTA) - This method remains the ‘gold standard’ to which enzyme immunoassays are compared, however, sensitivity depends on the cell line and the dilution titre used.
2. Enzyme Immunoassay (EIA) - A rapid and easy-to-use alternative to cell cytotoxicity. Toxin is detected using antibodies coated on to a membrane or a microtitre plate.
3. Real-time PCR - the use of molecular techniques to detect *C. difficile* infections remains limited.

Why consider culture for CDI diagnosis?

Culture is a sensitive method which is essential in laboratory diagnosis of CDI: to investigate patients with severe disease; for antibiotic susceptibility; for epidemiological studies in the case of outbreaks; and for suspected cases that have produced a negative stool result.

Culture of *C. difficile* is carried out on a selective medium, such as CCFA (Cefoxitin Cycloserine Fructose Agar), CDMN (C. difficile Moxalactam Norfloxacin), or Brazier’s CCEY (Cefoxitin Cycloserine Egg Yolk). Plates are incubated anaerobically at 37°C for 24-48 hours. Presumptive identification is made by examining colony morphology, the characteristic horse manure odour, positive Gram stain and yellow/green (chartreuse) fluorescence under UV light (365nm).

Biochemical testing can be carried out on presumptive colonies using an identification strip such as RapID™ ANA II.

Alcohol shock can be used to select bacterial spores, especially if using a less selective medium.

Treatment

Ceasing administration of the offending antibiotic therapy in patients with CDI symptoms should resolve the diarrhoea in a couple of days without the need for further treatment. In the case of elderly or fragile patients, those with severe or persistent symptoms and in circumstances where ongoing antibiotic therapy cannot be stopped, treatment must be prescribed. The main treatment for CDI is metronidazole. Patients not responding to this treatment may then be prescribed vancomycin.

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- **Clostridium difficile Agar Base** CM0901B
- **Clostridium difficile Selective Supplement** SR0098E
- **Clostridium difficile Moxalatam Norfloxacin Selective Supplement (CDMN)** SR0173E
- **Clostridium difficile Selective Agar (CCFA)** (UK) PB0218A (rest of Europe) PB5054A
- **Brazier's Clostridium difficile Selective Agar** (UK) PB1055A (rest of Europe) PB5191A
- **Anaerobic Jar 3.5 litre** HP0011A
- **Anaerobic Gas Pack 3.5 litre** BR0038B
- **AnaeroJar™ 2.5 litre** AG0025A
- **AnaeroGen™ 2.5 litre** AN0035A
- **AnaeroGen™ 3.5 litre** AN0036A
- **AnaeroGen™ Compact** AN0010C
- **AnaeroGen™ W-Zip Compact** AN0010W
- **C. difficile Test Kit** DR110CA
- **Xpect™ C. difficile Toxin A/B** R24650
- **ProSpecTTM C. difficile Toxin A/B Microplate Assay** R244596
- **RapID™ ANA II Panel** RB311002
- **PRO Discs w/ Reagent** R211357

*not CE marked + not available in some territories
Sample Collection
Liquid or unformed stools, fresh or stored ≤72hrs at 2-8°C

**Culture**

(Optional: alcohol shock treatment)

Inoculate selective medium

- C. difficile Selective Agar (CCFA)
  order code PB5054A / PB0218A
- Brazier’s C. difficile Selective Agar
  order code FBS191A / FBS195A
- C. difficile Selective Agar (CCFA)
  order codes CM0601B & SR0069E + 5% blood
- C. difficile Moxalam Norfloxacin
  (CCMN) Agar
  order codes CM0601B & SR0173E + 5% blood

Incubate anaerobically at 35-37°C for 24-48 hrs

AnaeroJar & AnaeroGen 2.5 litre
order codes AG0025A & AN0025A

Anaerobic Jar & AnaeroGen 3.5 litre
or Anaerobic Gas Pack plus catalyst
order codes: HP0011A & AN0035A or
BR0038B plus BR0042A

AnaeroGen Compact
order code AN0010C

AnaeroGen W-Zip Compact
order code AN0010W

**Presumptive Clostridium difficile**

Suspect colony

- C. difficile Test Kit
  order code DR1107A
- PRO Discs w/ reagent**
  order code R211357

**Toxin Detection**

- Xpect C. difficile Toxin A/B
  order code R24650
- ProSpecT C. difficile Toxin
  A/B Microplate Assay*
  order code R244596

**Optional**

Biochemical Identification: RapID ANA II* order code RB031002
Culture: for epidemiological studies; to investigate patients with severe disease; for suspect cases with negative stool results; and for antibiotic susceptibility testing

Susceptibility Testing: order codes for M.I.C.Evaluator™ strips:
- Penicillin G MA0103D&F / MA0101D&F
- Metronidazole MA0103D&F
- Imipenem MA0115D&F
- Amoxicillin/Clavulanic acid MA0107D&F

**References**


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Your local Oxoid representative will be pleased to provide further information on any product within our C. difficile Portfolio and our technical support team is available to give advice on product usage.

* not CE marked  + not available in some territories

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