Challenge M241-4

May 2024

Wound: *Clostridium perfringens* – Ungraded challenge

HISTORY

A simulated wound sample collected from a 23 year old in-patient, car crash victim was sent to category A laboratories. Participants were expected to isolate and report *Clostridium perfringens*

CMPT QA/QC/STATISTICS

All simulated wound samples are produced at CMPT according to CMPT internal protocols. The sample contained a pure culture of *Clostridium perfringens*.

The samples are assessed for homogeneity and stability using in-house quality control methods and random selection of samples before and during production, and post sample delivery. The number of random samples selected is 15% of the total production batch.

The sample lot showed heterogeneity in the viability of the organism, with 43% of samples showing viability up to 12 days while the rest showed a much shorter viability.

Organism identification was confirmed by a reference laboratory.

MAIN EDUCATIONAL POINTS from M241-4

1. Traumatic injury can result in introduction of soil organisms including *Clostridium* species into wounds.
2. A Gram stain result of gram positive bacilli suggestive of *Clostridium* would provide the clinician with valuable information that could save the patient.

All challenge components have in-house assigned values based on the most clinically appropriate result; the most clinically appropriate result is determined by expert committee evaluation. No further statistical analysis is performed on the results beyond that described under "Suitability for grading."

Grading

This challenge is Ungraded.

SURVEY RESULTS

Reference laboratories: 3/12 labs reported *C. perfringens*, 1 lab reported *S. epidermidis*, 8 labs reported no growth, 1 lab reported it does not process this type of sample - UNGRADED due to lack of consensus

Participants: only 9 participants were able to grow the organism and identify it as *C. perfringens* (Table 1).

Table 1. Identification results

Reported	Total	Grade
Clostridium perfringens	9	ungraded
Heavy Anaerobic isolate, refer, we do not identify anaerobic isolates in our lab.	1	ungraded
anaerobic gram positive bacillus, referred to reference lab for identification and susceptibility testing.	1	ungraded
No growth/negative culture/ no aerobic or anaerobic growth/Aucune croissance dans la plaie (no growth in wound)	25	ungraded
Gram positive bacilli failed to grow in aerobic and anaerobic culture for identification	5	ungraded
Gram negative Bacilli seen in gram stain failed to grow in aerobic and anaerobic culture. CMPT was not able to send us a repeat sample.	5	ungraded
Gram variable bacilli - failed to grow in culture	1	ungraded
Cutibacterium acnes	1	ungraded
Staphylococcus epidermidis from broth subculture only	1	ungraded
sample not normally processed	2	ungraded
Total	**51**	

Suitability for Grading

A challenge is considered suitable for grading if agreement is reached by 80 percent of selected reference group and at least 50 percent of the participants.

Because of the short viability in most of the samples sent, laboratories had difficulty isolating the organism, therefore, this challenge was ungraded.

COMMENTS ON RESULTS

Due to the short viability of this organism, only 9 labs were able to correctly identify *C. perfringens*. Two labs were able to grow the organism but would refer for identification. When referring to another laboratory, the report should include sufficient descriptive information to provide the clinician some guidance while awaiting a definitive identification.

Multiple laboratories, who did not obtain growth, performed direct smears from the samples with results ranging between gram positive, gram negative and gram variable bacteria. Anaerobes are easily over-decolorized. However, the distinctive morphology of large box car shaped rods should raise suspicion of *Clostridium perfringens* and a repeat Gram should be performed.

ISOLATION AND IDENTIFICATION

Clostridium is a genus of spore-forming, typically large gram positive rods but often over decolorize. Some species may appear gram negative even with minimal decolorization, notably *C. ramosum, C. innoculum,* and the *C. clostridioforme* group. Classically, *C. perfringens* appears as large, box-car shaped, gram positive rods with blunt ends.[1] The spores appear as clearings in the Gram stain and may assume terminal, sub-terminal, or central positions within the cells. *C. perfringens* and *C. ramosum* do not readily form spores but will sporulate at incubation temperatures below those required for optimal growth, usually 30°C.[2]

Complete identification should be attempted on isolates from sterile sites or from serious infections. Species identification is important as *C. perfringens* is the most frequent organism associated with gas gangrene, and can be extraordinarily aggressive. [1] Some species are notably associated with gastrointestinal malignancy (*C. septicum*) and should prompt further workup when isolated in blood cultures or systemic infections. *C. ramosum, C. innocuum*, and *C. clostridioforme* may be resistant to commonly used antibiotics. *C. innocuum* has an intrinsic resistance to vancomycin which is of note as a gram positive organism.

Clostridium species grow well on commercially available blood agar and growth is often rapid: *C. perfringens* can form colonies overnight or in as little as 6hs. [1] When clostridia are suspected in wound or abscess specimens, egg yolk agar may also be inoculated.

Speciation is largely based on morphology, location of spores and formerly biochemical reactions and gas-liquid chromatography identification of fermentation products were important, [1,2] although MALDI-TOF has also proven to be useful for rapid identification of *C. perfringens*.[3] *C. perfringens* is rather unique amongst the clostridia, as its colonies exhibit double zone hemolysis; that is, a small zone of beta-hemolysis and a larger one of partial hemolysis. The reverse CAMP test is positive. [1] Growth on egg yolk agar is positive for lecithinase and negative for lipase.[1]

ANTIMICROBIAL SUSCEPTIBILITY

C. perfringens is susceptible to penicillins, β-lactamase inhibitor combinations, carbapenems and metronidazole. [1] Most isolates are susceptible to clindamycin and moxifloxacin.[4]

CLINICAL RELEVANCE

Traumatic injury can result in introduction of soil and fecal organisms, including *Clostridium* species into wounds. Highly soiled open fractures or wounds are at high risk for clostridial infection.[2] Infections and related tissue damage result from the combination of injury and microbial virulence features, including exotoxins. *Clostridium* species account for 30% of all anaerobic organisms recovered from post-traumatic wounds.[5–8]

Commonly the Gram stain of tissue or fluid infected with *Clostridium perfringens* demonstrates many characteristic gram positive bacilli, often with reduced numbers of polymorphonuclear cells (PMNs) many of which are damaged.[9] *Clostridium* species produce a range of toxins.[1] The α toxin of *C. perfringens* is a phospholipase C that is both hemolytic and cytotoxic to platelets and leukocytes due to its ability to cleave sphingomyelin and the phosphoglycerides present in eukaryotic cell membranes.[10] The *C. perfringens* θ toxin (perfringolysin O) is similarly cytolytic for PMNs, especially in high concentration. [10]

C. perfringens, the species most frequently encountered in infections, does not normally sporulate in tissue, and thus the gram positive rods seen on Gram stain of tissue or other patient specimens do not usually exhibit sporulation. Instead, the classic appearance is that of large, boxy gram positive rods with blunt ends, and may be encapsulated in smears from wounds. [1]

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