

Challenge M244-2

February 2025

Eye: *Bacillus cereus*

HISTORY

A simulated eye swab sample collected from a 39 year old stone worker with a painful eye was sent to category A laboratories. Participants were expected to isolate and report *Bacillus cereus*.

CMPT QA/QC/STATISTICS

All simulated eye swab samples are produced at CMPT according to CMPT internal protocols. The sample contained a pure culture of *Bacillus cereus*.

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 challenge sample lot was confirmed to be homogeneous and stable for 15 days from shipping day. Organism identification was confirmed by a reference laboratory.

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."

SURVEY RESULTS

Reference laboratories

Identification: 11/12 (92%) processing labs reported *Bacillus cereus* ± group, 1 lab reported skin flora. One lab indicated it does not normally process this type of sample

Participants

Identification: 31/48 (65%) reporting laboratories reported *Bacillus cereus*; 11 participants reported *Bacillus* species; 2 reported gram positive bacilli and 4 reported normal flora (Table 1).

MAIN EDUCATIONAL POINTS from M244-2

1. The spores of *B. cereus* are commonly found in soil and are often associated with burn wounds in persons who work outside. Spores of the micro-organism gain entry to the wound if the burned person rolls in the dirt in an attempt to smother the flames.
2. Beta-hemolysis is an important primary determinant to separate *B. cereus* from other common *Bacillus* species pathogens, particularly *B. anthracis*.
3. Even reporting only a *Bacillus* species in an patient with this painful eye (clinical signs of endophthalmitis will be helpful for early treatment.

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.

Organism identification was correctly performed by at least 80 percent of reference laboratories and greater than 50 percent of all laboratories and was thus, determined to be suitable for grading.

Grading

Maximum grade: 4

Reporting *Bacillus cereus* or *Bacillus* species, refer or not anthracis was graded 4

Table 1. Identification results

Reported	Total	Grade
<i>Bacillus cereus</i> ± group	29	4
<i>Bacillus cereus</i> (complex)	1	4
<i>Bacillus</i> species, refer	7	4
<i>Bacillus</i> species, not <i>Bacillus anthracis</i> , refer	1	4
gram positive bacilli, refer ± spore-forming ± aerobic	4	3
Bâtonnet gram positif (aspect compatible avec <i>Bacillus</i> sp), snnp, contact lab for special requests	2	3
Skin flora ± mixed	3	0
Heavy growth of normal conjunctival flora. No pathogens isolated.	1	0
no report	1	0
sample not normally processed	2	ungraded
Total	51	

COMMENTS ON RESULTS

Over 85 % of laboratories recognized that this isolate was potentially important as a pathogen in this case – where an microorganism such as a *Bacillus* could cause in infection in a stone worker – where contact with soil can contain spores of *Bacillus* species. All these laboratories received a grade of 4,(add comment on the one graded 3) The two labs that recognized the isolate as likely a *Bacillus* even though they don't normally process eye specimens received a grade of 3.

It would be helpful if not identifying the actual species, if the isolate was identified and reported as a beta-haemolytic strain, which in this case should point to *B. cereus*.

ISOLATION AND IDENTIFICATION

B. cereus group organisms are indistinguishable in Gram stained or spore-stained smears, appearing as large, regular gram positive rods with square ends. Oval, sub-terminal spores that do not distend the cell are seen in cultures older than 24 hr and may be distinguished in the primary Gram smear..

The species of the *B. cereus* group traditionally include *B. cereus*, *B. thuringiensis*, *B. anthracis* and *B. mycoides*. The latter two species are non-hemolytic. All species produce lecithinase. Several other species that were formerly considered part of the *B. cereus* group are now considered separate species. One of these is *B. weihenstephanensis*, which was formerly considered as psychrotolerant *B. cereus*.¹ *Bacillus* species other than the *B. cereus* group rarely require identification in the clinical laboratory, unless isolated from sterile fluids.

Isolates can be identified using commercial systems, such as the Vitek Bacillus card or the Biolog system, by FAME (MIDI system) or by MALDI-TOF.

Any isolate of the *B. cereus* group should be evaluated to exclude *B. anthracis*. β -hemolytic colonies essentially rule out *B. anthracis*. *B. cereus* is a well-recognized opportunistic pathogen and should not be dismissed as normal flora.

ANTIMICROBIAL SUSCEPTIBILITY

Although antimicrobial susceptibility was not expected, It is well-known that *B. cereus* can produce beta-lactamases, and therefore penicillin and cephalosporin agents are less effective. Most isolates are susceptible to fluoroquinolones (ciprofloxacin and levofloxacin) rifampicin, vancomycin and gentamicin. Carbapenems such as imipenem may be effective.

Since *B. cereus* is more commonly associated with food borne illness (e.g. undercooked rice that allows the spores to survive and then become ingested), antimicrobial susceptibility testing is not necessary in such instances.

CLINICAL RELEVANCE

Endophthalmitis may be infectious or non-infectious. Infectious endophthalmitis may be a result of endogenous or exogenous sources. Post-traumatic endophthalmitis make up 25%–30% of all infectious endophthalmitis cases² and the organisms involved can be endogenous flora from the periorbital region that gain access into the open wound or environmental organisms that are directly inoculated into the eye during trauma.

Gram positive bacteria are responsible for the majority of the posttraumatic endophthalmitis with approximately 20% of cases attributed to *Bacillus* species.^{2,3}

Endophthalmitis cases present acutely and are medical emergencies, as delay in treatment may result in permanent vision loss.⁴

Compared to other pathogens associated with any type of endophthalmitis, the bacterium *B. cereus* is an exceptionally virulent danger to the eye. *B. cereus* grows rapidly in the eye and possesses a complex cohort of virulence factors, making the window for successful therapeutic intervention relatively short.⁵

Bacillus infections can result in a total loss of vision with enucleation. The most common species of *Bacillus* associated with posttraumatic endophthalmitis are *B. cereus*, *B. subtilis*, and *B. licheniformis*.^{4,6}

Treatment should be aggressive, with vitrectomy, intravitreal antibiotics, and systemic therapy.⁴

REFERENCES

1. Turenne CY, Snyder JW, Alexander DC. *Bacillus* and other aerobic endospore-forming bacteria. In: Jorgensen, J. H. et al., ed. Manual of clinical microbiology. Vol 1. 10th ed. Washington, DC.: ASM; 2015:441.
2. Bhagat N, Nagori S, Zarbin M. Post-traumatic infectious endophthalmitis. *Surv Ophthalmol*. 2011;56.
3. Long C, Liu B, Xu C, Jing Y, Yuan Z, Lin X. Causative organisms of post-traumatic endophthalmitis: A 20-year retrospective study. *BMC Ophthalmology*. 2014;14(1):34.
4. Durand ML. Endophthalmitis. *Clin Microbiol Infect*. 2013;19(3):227-234.
5. Mursalin MH, Livingston ET, Callegan MC. The cereus matter of bacillus endophthalmitis. *Exp Eye Res*. 2020;193:107959.
6. McMullen AR, Burnham CA. Be serious: Posttraumatic endophthalmitis. *Clin Chem*. 2016;62(1):37-39.