Challenge M241-3

May 2024

Sputum: *Haemophilus influenzae*

HISTORY

A simulated sputum sample collected from a 75 year old male with exacerbation of COPD was sent to category A laboratories. Participants were expected to isolate and report *Haemophilus influenzae*

CMPT QA/QC/STATISTICS

All simulated sputum samples are produced at CMPT according to CMPT internal protocols. The sample contained a mixed culture of *Haemophilus influenzae* and *Streptococcus viridans*.

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 9 days.

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: 13/13 (100%) labs reported *H. influenzae* ± normal/usual/mixed oropharyngeal flora.

Participants: 47/50 (94%) participants reported *H. influenzae* ± normal/usual/mixed oropharyngeal flora. One lab reported *Haemophilus* species, refer, another lab reported gram negative coccobacilli, and one reported *Streptococcus pneumoniae* (see Table 1).

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.

MAIN EDUCATIONAL POINTS from M241-3

1. *Haemophilus influenzae* is an important cause of exacerbations of chronic bronchitis, and the most commonly isolated bacterial cause.
2. COPD patients who acquire new strains of *H. influenzae, Streptococcus pneumoniae* and *Moraxella catarrhalis*, are more prone to developing exacerbation episodes with bacterial infection.

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 *H. influenzae* was graded 4.

COMMENTS ON RESULTS

Participants performed very well on this challenge, with almost all labs reporting *Haemophilus influenzae* – these labs were graded 4.

A single lab reported *Haemophilus* species, refer – this lab was graded 3 as identification of *Haemophilus influenzae* should be possible in Category A laboratories. A single lab reported gram negative coccobacilli without any additional information and was graded 1.

A single lab reported *Streptococcus pneumoniae*, and failed to recognize/identify the *Haemophilus,* and was thus graded 0.

Table 1. Identification results

Reported	Total	Grade
Haemophilus influenzae ± normal/usual/mixed oropharyngeal flora	47	4
Haemophilus species, refer	1	3
4+ gram negative coccobacilli	1	1
Streptococcus pneumoniae	1	0
sample not normally processed	1	ungraded
Total	**51**	

ISOLATION AND IDENTIFICATION

Haemophilus influenzae is a small, non-motile, gram negative coccobacillus. In Gram-stained clinical specimens they can present a variable morphology ranging from small coccobacilli to long filaments.[1,2]

Haemophilus species are facultatively anaerobic. *H. influenzae* requires the presence of both, X (hemin) and V (nicotinamide adenine dinucleotide – NAD) factors, whereas most other species only require one factor.[2]

Satellitism around colonies of hemolytic *Staphylococcus aureus* (a source of V factor) is typically observed for *H. influenzae*.

The porphyrin test, which depends on the ability of an organism to synthesize fluorescent heme precursors from alpha aminolevulinic acid (ALA), is a rapid and reliable test to help distinguish *H. influenzae* (porphyrin negative) from *H. parainfluenzae* (porphyrin positive). The organism is accurately identified using MALDI-TOF.

H. influenzae is divided into typeable and non-typeable strains based on the presence or absence of a polysaccharide capsule. Six serotypes (a to f) have been described based on the antigenically distinct capsular polysaccharide types. *H. influenzae* type b strains are important invasive pathogens in humans while the non-typeable strains are the main cause of respiratory infections.[3]

ANTIMICROBIAL SUSCEPTIBILITY

A number of antibiotics are effective against *H. influenzae*, including ampicillin, second and third generation cephalosporins, and trimethoprim-sulfamethoxazole. β -lactamase testing is a useful rapid test, but if negative should be followed by further testing.

Most isolates of *H. influenzae* that are resistant to ampicillin and amoxicillin produce a TEM-type β-lactamase which is usually detectable by a direct β-lactamase test. These strains are resistant to ampicillin and amoxicillin but remain susceptible to amoxicillin-clavulanic acid.

Decreased PBP3 affinity has also been reported in *H. influenzae* as a resistance mechanism. These strains are rare in Europe and North America but they are prevalent in Japan and Korea.[4] These strains are referred to as beta lactamase negative ampicillin resistant (BLNAR) and are considered to be resistant to amoxicillin-clavulanic acid and second generation cephalosporins such as cefuroxime.

Although susceptibility testing has been historically performed using Haemophilus test medium (HTM) in 5% CO_2 [4] CMPT has learned that there have been recent collaborations between two of the major AST breakpoint groups (CLSI and EUCAST), regarding standardizing the Haemophilus susceptibility testing medium to Mueller-Hinton- Fastidious (MH-F) for both micro-dilution testing and disc diffusion, currently used by EUCAST.

When this change has been made public, CMPT will inform our users with additional information regarding quality control considerations. For disc diffusion testing, this medium is MH agar supplemented with 5% (v/v) mechanically defibrinated horse blood and 20 mg/L β-NAD ('Mueller–Hinton fastidious', MH-F) for fastidious organisms.[7] For broth microdilution, the medium is cation-adjusted Mueller Hinton broth (CAMHB) supplemented with 5% lysed horse blood and 20 mg/L β-NAD.[7]

CLINICAL RELEVANCE

Chronic obstructive pulmonary disease (COPD) is associated with periodic flare-ups of respiratory symptoms, which are referred to as "exacerbations". As the disease progresses, the flare ups occur on a background of chronic symptoms. The airways become chronically colonized by bacteria.

Non typeable *Haemophilus influenzae* (NTHi), which is associated with as much as 30% of acute exacerbations, is one of the more common isolates from sputum in exacerbations, although it can often be isolated from sputum from as many as 30% of patients with COPD in between exacerbations.[6] It is suggested that NTHi contributes to the ongoing inflammation and has been seen, using immunofluorescence, invading the bronchial tissues during exacerbations.

Patients who acquire new strains of *H. influenzae*, as well as *Streptococcus pneumoniae* and *Moraxella catarrhalis*, are more prone to develop COPD exacerbation episodes with bacterial infection.[5] The ongoing inflammation, release of cytokines and damaging enzymes from the inflammatory cells result in progressive airway obstruction and alveolar damage. This process results in progressive damage to the lung.[5]

REFERENCES

1. Murphy TF. *Haemophilus* Infections. In: Mandell D Bennett, ed. Vol 2. 6th ed. Elsevier; 2005:2661.
2. Gonnzalez MD, Ledeboer, N.A. *Haemophilus*. In: Carroll KC et al, ed. *Manual of Clinical Microbiology*. Vol 2. 13th ed. ASM; 2023:714.
3. King P. *Haemophilus influenzae* and the lung (*Haemophilus* and the lung). *ClinTranslMed*. 2012;1(1):10-10. doi:10.1186/2001-1326-1-10;
4. Inoue S, Watanuki Y, Miyazawa N, et al. High frequency of beta-lactamase-negative, ampicillin-resistant strains of *Haemophilus influenzae* in patients with chronic bronchitis in Japan. *JInfectChemother*. 2010;16(1):72-75. doi:10.1007/s10156-009-0020-5;
5. Eldika N, Sethi S. Role of nontypeable *Haemophilus influenzae* in exacerbations and progression of chronic obstructive pulmonary disease. *Current Opinion in Pulmonary Medicine*. 2006;12 (2):118. doi:10.1097/01.mcp.0000208451.50231.8f
6. Short B, Carson S, Devlin A, et al. Non-typeable *Haemophilus influenzae* chronic colonization in chronic obstructive pulmonary disease (COPD). *Critical Reviews in Microbiology*. 2021;47(2):192-205
7. EUCAST. January 2022. https://www.eucast.org/fileadmin/src/media/. Media preparation for EUCAST disk diffusion testing and for determination of MIC values by the broth microdilution method. Version 7