Microbiological processing of respiratory specimens from patients with cystic fibrosis

RECOMMENDATIONS OF THE CLINICAL SUBCOMMITTEE OF THE MEDICAL/SCIENTIFIC ADVISORY COMMITTEE OF THE CANADIAN CYSTIC FIBROSIS FOUNDATION

THE BACTERIOLOGY OF CYSTIC FIBROSIS (CF) PULMONARY infections has been the focus of attention for decades (1). Nevertheless, many controversies still exist concerning the rates and clinical implications of colonization with organisms such as *Pseudomonas aeruginosa* and *Pseudomonas cepacia*. To assess appropriately the impact of colonization with these organisms, the microbiological techniques used in processing the respiratory specimen from patients with CF must be capable of detecting all of the common organisms associated with CF such as *Staphylococcus aureus*, *Haemophilus influenzae*, *P aeruginosa*, *P cepacia*, and other *Pseudomonas* species as well as related organisms such as xanthomonas. Establishment of a minimum protocol for assessment of sputum and throat swabs from CF patients all across Canada would allow for comparison of colonization rates between centres and regions and increase the sample size for validation of clinical associations, thus providing the foundation for epidemiological studies. These guidelines were therefore developed by the Clinic Subcommittee of the Canadian Cystic Fibrosis Foundation based upon recommendations from the Canadian Association of Medical Microbiologists (2).

LABORATORY ACCREDITATION AND PROFICIENCY TESTING

All laboratories in Canada which perform work for CF clinics need to be fully accredited and subscribe to proficiency testing programs which allow laboratories to track their capability of reliably identifying the presence of organisms such as *P cepacia* in sputa (3). Furthermore, on a regular basis, proficiency test specimens should include in their panels the commonly found organisms seen in patients with CF (eg, *S aureus*, *H influenzae*, *P aeruginosa*, *P cepacia*).

RESPIRATORY SPECIMENS FOR CULTURE

Sputum: In patients with CF, unlike some other pulmonary diseases, there is a strong concordance between the organisms detected in sputum and those detected in specimens obtained at thoracotomy (4). Thus sputum is a useful and – for older patients and those capable of producing sputum – a relatively easily obtainable specimen for determining the lower tract pathogens.

Throat swabs/auger suction secretions: In young patients, throat swabs may be the only specimen readily available. Although auger suction is often used as a surrogate for a deep pharyngeal specimen, the aspirate

Clinic Subcommittee – Members: Noni MacDonald (chairperson), Department of Pediatrics, Children's Hospital of Eastern Ontario, Ottawa, Ontario; Alphonse Jeanneret, Service de Pneumologie, Hôtel-Dieu de Montréal, Montréal, Québec; Ian Mitchell, Alberta Children's Hospital, Calgary, Alberta; Edena Nakielna, University Hospital, Shaughnessy Site, Vancouver, British Columbia; Joseph Reisman, Hospital for Sick Children, Toronto, Ontario; Georges Rivard, Centre Hospitalier de l'Université Laval, Ste-Foy, Québec; William Wilson, Children's Hospital at Chedoke-McMaster, Hamilton, Ontario. **Consultant:** David P Speert, British Columbia's Children's Hospital, Vancouver, British Columbia. **Liason Member:** Ian MacLusky, Hospital for Sick Children, Toronto, Ontario

Correspondence and reprints: Dr Noni E MacDonald, Director, Cystic Fibrosis Service, Children's Hospital of Eastern Ontario, 401 Smyth, Ottawa, Ontario, K1H 8L1. Telephone (613) 737-7600

still remains a sample of upper, not lower, tract secretions. Unfortunately, for these two specimens the predictive value for determining lower tract pathogens from the upper tract isolates is limited especially with respect to *H influenzae* and *S aureus* which are often part of the normal flora of the upper tract. Isolation of pseudomonas from the upper tract may be more useful in the sensitivity pattern of possible lower tract pathogens as these organisms are not upper respiratory commensals under normal circumstances.

Sinus aspirations: The bacterial species recovered from sinus aspirates from patients with CF are not dissimilar from those found in sputa, eg, pseudomonas and *H influenzae* (5). However, there is no concordance between the bacterial species recovered from the sinus and the predominant bacterial species in the nasopharyngeal, throat, or sputum culture (5). Nonetheless, bacterial infection of the sinuses presents a potential clinical problem in patients with CF.

MICROBIOLOGICAL PROCESSING OF CF SPUTUM

Sputum grading and Gram stain: All properly collected sputa should be graded (6) to ensure that specimens highly contaminated with saliva will be detected. The Gram stain may also assist in determining if special testing, above and beyond that which is routinely recommended, may be helpful. For example, depending upon the clinical findings, additional tests for agents such as *Mycoplasma pneumoniae*, *Bordetella pertussis*, *Legionella* species, *Aspergillus* species, atypical mycobacteria, respiratory syncytial virus, adenovirus, parainfluenza virus, influenza virus, and rhinovirus may be indicated.

Sputum liquefaction and quantitative bacteriology: Given the high viscosity of sputa from patients with CF, especially those with advanced disease, if quantitative bacteriology is desired sputa must first be liquefied with agents such as *n*-acetylcysteine (Mucomyst; Bristol) or dithiothreitol (Sputolysin) (7,8). Mechanical methods of liquefaction are time consuming and do not provide major advantages over chemical methods. There is controversy over the potential antibacterial effect of the chemical agents which may be related to contact time (9). Quantitative methods, which use serial dilutions of homogenized sputum, may enhance detection of some bacteria and variant strains of pseudomonas (7,8) and are sometimes used to assess the impact of antimicrobial therapy during research studies. Semi-quantitative cultures with careful assessment are less time consuming and are considered adequate in most settings.

Sputum culture – Selective and differential media: Selective and/or differential media (Table 1) can enhance the probability of detecting specific organisms. Since using all of these media adds to the cost of processing, many laboratories use a MacConkey medium or equivalent for preliminary differentiation of pseudomonas. Most patients with predominating

TABLE 1

Solid media recommended for enhanced recovery of probable bacterial pathogens in cystic fibrosis sputa

Pathogen	Medium
<i>Pseudomonas aeruginosa</i>	MacConkey
<i>Pseudomonas cepacia</i>	Polymixin B-MacConkey (modified) OFFBL PC
<i>Haemophilus influenzae</i>	Chocolate-anaerobic incubation
<i>Staphylococcus aureus</i>	Mannitol-salt agar Colistin-nalidixic acid agar

P cepacia will be identified if semi-quantitative methods are used. However, patients with mixtures of pseudomonas where *P cepacia* is not predominant may be missed. Detection of carriage of *P cepacia* can be enhanced with selective media (10,11). Given that clinical decisions such as cohorting (12) are based upon the presence or absence of *P cepacia*, optimal methods to detect this organism need to be used.

The detection of *H influenzae* can be facilitated by suppressing *P aeruginosa* by anaerobic incubation of the specimen on chocolate agar. Chocolate-bacitracin agar is less useful since this will not adequately suppress *P aeruginosa*. Mannitol-salt agar, even the thymine-dependent variant, can be used to detect *S aureus*.

Detection of *P cepacia*, *H influenzae*, *S aureus* and other pseudomonas using the above noted selective media and culture conditions (MacConkey, chocolate blood [anaerobic conditions], mannitol-salt, *P cepacia* selective media) can be optimized with prolonged incubation for up to three days. This allows slower growing colonies to become more apparent and may be particularly important if the patient is receiving antibiotics.

Antibiotic susceptibility testing of isolates from sputa: Since clinical decisions regarding antibiotic therapy for pulmonary exacerbations are guided by the organisms present in the sputum as well as their antimicrobial susceptibilities, methods for determining the following resistances need to be available in laboratories processing sputum specimens from patients with CF:

- **Pseudomonas:** Susceptibility to tobramycin, gentamicin/netilmicin, amikacin, ciprofloxacin, cotrimoxazole, colistin, ticarcillin, piperacillin, ceftazidime, cefsulodin, cefoperazone, imipenem and ticarcillin/clavulanic acid. The specific agents tested among the latter will be dictated by local patterns of practice and discussions between the laboratory and CF clinician. Occasionally, multiresistant pseudomonas will emerge as the predominant pathogens. When therapeutic options are limited (based upon conventional testing), more elaborate

combination testing studies may be helpful (13). This type of testing, eg, checkerboards, solid media, may detect synergistic versus antagonistic combinations (14). Such testing is not widely available, but is done in some tertiary care CF centres both in Canada and the United States. Interpretation and the clinical value of this type of testing is still unclear for patients with cystic fibrosis.

- *S aureus*: Beta-lactamase testing, detection of methicillin-resistant strains, differentiation of and susceptibility testing of thymine-dependent strains.
- *H influenzae*: Beta-lactamase testing and detection of beta-lactamase negative ampicillin-resistant strains.

If an automated susceptibility testing device is used, false test results may occur if the organism is slow growing (eg, *P cepacia* may appear falsely sensitive to agents such as some third generation cephalosporins) or the recommended inoculum is not attained (eg, methicillin-resistant *S aureus* and *P aeruginosa* with class I beta-lactamase resistance may appear falsely sensitive to some expanded spectrum beta-lactam antibiotics and *H influenzae* may appear to be falsely resistant to expanded spectrum cephalosporins) (15). Laboratories must ensure that reliable methods are being used for reproducible susceptibility testing of CF pathogens. While proficiency testing can help ensure that most of these methods are accurate and reliable, not all types of resistance problems are included in the programs.

MICROBIOLOGICAL PROCESSING OF NONSPUTUM RESPIRATORY SPECIMENS

Sinus aspirates should be processed in a manner analogous to sputum specimens with additional testing for anaerobes (5).

Throat swabs and auger suction secretions do not merit the same precise quantitative or semi-quantitative bacteriology as sputum methods due to the inherent imprecision of these specimens in reflecting lower tract pathogens. This is particularly true for *H influenzae* and *S aureus*. However, since the determination of the presence or absence of organisms such as *P cepacia* or other multiresistant pseudomonas may influence clinical decisions regarding cohorting and antimicrobial selection for exacerbations, care needs to be taken when processing these specimens.

GUIDELINE RECOMMENDATIONS

The Clinic Subcommittee suggests that all laboratories processing respiratory specimens from patients with CF, at a minimum:

- Need to be fully accredited and to belong to a proficiency testing program;
- Will grade and Gram stain sputum and process

using liquefaction or semi-quantitative bacteriology methods with selective media to ensure that common CF pathogens are detected, eg, *S aureus*, *H influenzae*, *P aeruginosa*, *P cepacia*, and other pseudomonas;

- Will ensure that reliable methods for susceptibility testing of CF pathogens isolated from sputum will be used;
- Will process sinus aspirates from such patients in a manner similar to sputum with the addition of culturing for anaerobes;
- Will process throat swabs and auger suction specimens from CF patients upon request such that the presence or absence of *P cepacia* or other multiresistant pseudomonas may be detected;
- In appropriate clinical situations, the laboratory may need to expand the testing of sputum and/or other respiratory specimens to include testing for fungi such as aspergillus, bacteria such as mycobacteria and mycoplasma, and viruses such as respiratory syncytial virus and influenza virus.

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