# Department of Microbiology
# Cystic Fibrosis Culture Procedure

## I. Principle

The disruption of the exocrine function is the basis of many of the clinical aspects of cystic fibrosis (CF) disease. Patients have a basic problem with the way ions and fluids are transported in and out of mucus-producing cells. Mucus secretions should be fluid and thin to lubricate and move through ducts and through various channels of the body. In CF patients, mucus secretions are thick, dry, and sticky so that they clog passages. When microorganisms enter the distal airways in the lungs of CF patients, they are not cleared. Instead of transient colonization seen in normal lungs, the organisms cause chronic infections, an exaggerated inflammatory response, and progressive bronchiectasis until the airways lose function and the patients suffer respiratory insufficiency. Chronic pulmonary infection is the primary cause of the early mortality associated with cystic fibrosis.

## II. Organism Role in CF Lung Disease

A. Organisms clearly associated with lung disease in CF patients.

1. ***Staphylococcus aureus***
   a. Recovered from ~50% of CF patients.
   b. Small colony variants can be isolated from 50% of the CF patients with *S. aureus*. These organisms produce small, non-hemolytic, non-pigmented colonies on BAP and chocolate agar.
   c. Mannitol salts agar supports these small colony variants and prevents overgrowth by *P. aeruginosa* and *B. cepacia*.
   d. Oxacillin-resistant strains are being seen with an increasing frequency (6-20% of strains).
2. ***Pseudomonas aeruginosa***
   a. Most important pathogen for CF lung disease, infecting ~60% of the entire CF population and 80% of adolescents and adults.
   b. *P. aeruginosa* infections can be seen as early as infancy.
   c. Initial strains are "rough", motile, and tend to be sensitive to many antibiotics.
   d. Chronic infection is usually with mucoid strains that are non-motile, grow poorly, may lose pigment production, and are frequently resistant to many antibiotics. These strains may be misidentified by commercial systems.
   e. The presence of mucoid strains of *P. aeruginosa* indicates the beginning of the chronic phase of infection – characterized by pulmonary exacerbations (fever, increased WBC count, increased sputum production, and decreased pulmonary function) requiring antimicrobial therapy. Lung function continuously declines, the organisms become increasingly resistant, and the patient eventually has cardiopulmonary failure.
   f. Resistant isolates can be sent for susceptibility testing, including in vitro synergy testing, to Dr. Lisa Saiman at Columbia University in NYC.


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**3. *Burkholderia cepacia***

a. *B. cepacia* contains 9 genomovars, or genomic species, that are collectively referred to as *B. cepacia* complex.
b. ~3% of American CF patients and ~15% of Canadian CF patients are colonized with *B. cepacia*.
c. Infection with *B. cepacia* is associated with increased rates of morbidity and mortality.
d. 20% of patients colonized with *B. cepacia* develop the "cepacia syndrome", characterized by a rapid decline in pulmonary function, frequent bacteremia, and lung failure.
e. Person-to person spread of the organism among CF patients has been documented; as a result, these patients are excluded from social events for CF patients and are rejected as lung transplant recipients at many CF centers due to potentially poor outcomes. Therefore, organism identification must be accurate.
f. Selective media must be used to isolate the complex from respiratory secretions. These media inhibit the growth of other potential pathogens in CF patients, including *P. aeruginosa*.
    - BCSA (*B. cepacia* Selective Agar): preferred medium; highest sensitivity and specificity
    - PC Agar (*Pseudomonas cepacia* Agar)
    - OFPBL Agar (Oxidative-Fermentative base, polymyxin B, Bacitracin, and Lactose Agar)
g. Unfortunately, it is difficult to phenotypically identify the different genomovars – only 2 of the 9 genomovars have a proven role in CF lung disease: *B. multivorans* (genomovar II) and *B. cenocepacia* (genomovar III).
h. In addition to the problem of differentiating the genomovars, the *B. cepacia* complex is difficult to separate from phenotypically similar organisms, including *B. gladioli*, *Ralstonia* spp., and *Pandoraea*. *B. gladioli* and *Ralstonia* spp. can grow on BCSA. There are no data to support the role of the latter organisms in CF lung disease. Misidentification of these apparently harmless saprophytes as *B. cepacia* may have profound medical and social consequences for the patient.
i. Definitive identification of *B. cepacia* genomovars requires molecular testing. The isolate should be sent to: Dr. J. J. LiPuma, University of Michigan Medical School in Ann Arbor, the Cystic Fibrosis Foundation *B. cepacia* Research Laboratory and Repository.
j. *B. cepacia* complex is typically resistant to virtually all antibiotics, including cephalosporins and aminoglycosides. Non-treated strains may be susceptible to piperacillin-tazobactam, ceftazidime, trimethoprim-sulfamethoxazole, and imipenem.

B. Organisms that have a secondary role in CF lung disease.
   1. ***Haemophilus influenzae***
      a. Recovered from ~15% of CF patients – typically children.
      b. Few data support a primary role for this organism in CF lung disease.
   2. ***Aspergillus fumigatus***
      a. Molds are frequently isolated from CF patients; only *Aspergillus* spp. are associated pulmonary symptoms in CF patients.
      b. The organism is associated with allergic bronchopulmonary aspergillosis which is diagnosed clinically rather than by culture.
      c. The reporting of molds in this patient group is of limited value.
   3. **Respiratory viruses (RSV, influenza virus)**

C. Organisms being isolated with an increasing frequency from CF patients, but their role in CF lung disease has yet to be clearly determined.
   1. ***Stenotrophomonas maltophilia* & *Alcaligenes xylosoxidans***
      a. Being seen with increasing frequencies in the adult CF population.
      b. Role in CF lung disease has not been established.
      c. Identification can be difficult – may be misidentified as *B. cepacia* complex. Some strains may grow on OFPBL and PC agars.
      d. Clinicians caring for CF patients do want to know if these organisms are present.
   2. ***Mycobacterium* species other than *tuberculosis***
      a. *M. avium* complex and rapidly growing mycobacterial species are recovered from up to 20% of CF patients.
      b. Stringent decontamination conditions must be followed to avoid contamination with *P. aeruginosa*. Sequential treatment with the standard *N*-acetyl cysteine-NaOH followed by oxalic acid treatment significantly increases the recovery of nontuberculous mycobacteria.


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## Cystic Fibrosis Respiratory Culture Work-up

Follow the procedure for the routine work-up of lower respiratory cultures, with the following exceptions (remembering that throat cultures from CF patients are handled just like sputum cultures):

1. Incubate all media (BAP, CHOC, MAC, Mannitol Salt, BCSA and/or PC) for 5 days, and examine daily.
2. Do not use canned comments for Q0 specimens. Potential pathogens should be worked up regardless of the number of squamous epithelial cells seen on the direct smear.
3. Identification of non-fermenting gram-negative bacilli may be performed by the use of Phoenix, API 20E, or the N/F TEK systems. Additional conventional testing may be needed for the accurate identification of some isolates. **Consult on Rounds**.
4. Antimicrobial susceptibility testing of non-fermenting gram-negative bacilli from CF patients by automated systems may be unreliable. Susceptibility testing should be performed by Kirby-Bauer.
5. Use the BCSA and PC media only to select for *B. cepacia*. *B. cepacia* may take 3 days to grow on selective medium and has a characteristic dirt-like odor. Do not report other organisms from this medium.
6. Bring all potential *B. cepacia* isolates up on Rounds. Only finalize a report of "*B. cepacia*" with Round's approval.
7. Suspected *B. cepacia* isolates must be identified by API 20E and conventional biochemicals, including sucrose, lactose, lysine, ornithine, and growth at 42°C. If the API 20E gives an identification of *Burkholderia* species, the following table can be used to help with speciation.

Table
Identification schema for bacteria that grow on a selective medium and produce an identification of Burkholderia species by a commercial system:

| Test | B. multivorans & genomovar VI | B. vietnamiensis & genomovars I, III, VII | B. stabilis | B. gladioli |
|---|---|---|---|---|
| Oxidase | Weak + | Weak + | Weak + | - |
| Sucrose oxidation | - | + | - | - |
| Lactose oxidation | + | + | + | - |
| Lysine oxidation | v | + | + | - |
| Ornithine decarboxylase | - | v/v/-/- | + | - |
| ONPG | + | + | - | + |
| Growth at 42 degrees C | + | v/v/+/v | - | + |


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8. PYR can help to separate *B. cepacia* (PYR-negative) from *Ralstonia* spp. (PYR-positive).
9. The first isolate of suspected *B. cepacia* complex from a CF patient will be sent to the *Burkholderia cepacia* Research Laboratory and Repository funded by the CF Foundation for confirmatory testing.

    Dr. John LiPuma
    *B. cepacia* Research Laboratory
    University of Michigan in Ann Arbor
    1150 W. Medical Center Drive
    8323 MSRB III, Box 0646
    Ann Arbor, Michigan 48109-0646
    Telephone: (734) 936-9767
    FAX: (734) 764-4279
10. The 2004 NCCLS M100-S14 tables for disk diffusion include new breakpoints for interpretation of limited drugs for *S. maltophilia* (levofloxacin, minocycline, and trimethoprim-sulfamethoxazole) and *B. cepacia* (ceftazidime, meropenem, minocycline). These agents can be reported by disk diffusion testing for these isolates.
11. Resistant isolates of *P. aeruginosa*, *B. cepacia*, and *S. maltophilia* can be sent to Dr. Lisa Saiman's laboratory at Columbia for susceptibility testing.
    CF Referral Center For Susceptibility & Synergy Studies
    College of Physicians & Surgeons of Columbia University
    650 West 168th Street
    Black Building 4/413
    New York, NY 10032
    Telephone: (212) 305-1991
12. Slide coagulase testing on staphylococcal isolates can NOT be performed from mannitol salt agar. The high salt content can cause autoagglutination, leading to false positive results. Also, it is important to note that, in addition to *S. aureus*, there are a number of coagulase-negative staphylococci that can utilize mannitol, causing acidification of the medium.
13. If the number of potential pathogens is more than 3, only identify and perform appropriate susceptibility testing on *P. aeruginosa*, *B. cepacia*, *S. maltophilia*, *H. influenzae*, and *S. aureus*. Just list all other potential pathogens.

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