# Questionnaire

of

the microbiological procedures
of respiratory specimens
from Cystic Fibrosis patients

Please mark with a cross or use the free text field.

Address of laboratory:

**Note:**

The questionnaire is based on the German microbiological infectious quality standards for airway infections in CF 2006 (MiQ) and on the protocols provided by the consultant laboratories for CF microbiology (Institute of Microbiology, Medical School Hannover, Germany; Max von Pettenkofer-Institute for Hygiene and Medical Microbiology, Munich, Germany).

**1.** Of **how many CF-patients** does your laboratory regularly take care concerning microbiological testing?

Number: _____

**2.** Do you use special **guidelines** for the microbiological testing of respiratory specimens from CF-patients?

○ Yes ○ No

What kind of? _____

**3.** What kind of **materials** from CF-patients do you process?

○ Sputum
○ Nasal swab
○ Throat swab
○ Bronchoalveolar lavage
○ Others: _____

**4.** Do you **homogenize** the sputum before processing?

○ Yes ○ No

With what kind of substance?
○ Dithiothreitol
○ NALC
○ Others: _____

**5.** Do you streak the original material **before** homogenizing and liquefaction?

○ Yes ○ No

On what kind of plate?
- ○ Columbia blood agar
- ○ MacConkey agar
- ○ Chocolate agar
- ○ Others: _____

**6.** Do you determine the **number of pathogens**?

○ Yes ○ No

Do you make serial dilutions for the determination of pathogen number?

○ Yes ○ No

How many steps of serial dilutions do you perform?
- ○ $10^{-1}$
- ○ $10^{-3}$
- ○ $10^{-4}$
- ○ $10^{-5}$
- ○ Others: _____

**7.** What are the **culture media und conditions** you use to detect the following pathogens?

| | | |
|---|---|---|
| **H. influenzae** | ○ Chocolate agar<br>○ with bacitracin<br>○ without bacitracin<br>○ with _____<br>○ Others: | Incubation<br>○ under $CO_2$<br>○ anaerobic |
| **P. aeruginosa** | ○ Tryptic Soy Agar<br>○ Pseudomonas Isolation Agar | ○ MacConkey agar<br>○ Cetrimid agar<br>○ Others: |
| **B. cepacia** | ○ BCSA<br>○ OFPBL agar | ○ PC agar<br>○ Others: |
| **S. aureus** | ○ Columbia blood agar<br>○ Mannitol salt agar<br>○ BHI-NaCl-Agar<br>○ Baird-Parker agar<br>○ MRSA selective agar | ○ S. aureus ID agar<br>○ CHROMagar Staph aureus<br>○ Polymyxin/Nalidixic acid agar<br>○ Others: |
| **S. maltophilia** | ○ MacConkey agar<br>○ VIA agar | ○ Meropenem agar<br>○ Others: |
| **A. xylosoxidans** | ○ MacConkey agar | ○ Others: |
| **Fungi** | ○ Sabouraud agar<br>○ Kimmig agar | ○ Others: |

**8.** How long do you **incubate** the different **culture media** and at **which temperatures**?

| | **Time** | | | | **Incubation** | | | |
|---|---|---|---|---|---|---|---|---|
| **Pathogens** | 24h | 48h | 5d | Other | 30°C | 32±1°C | 36±1°C | Other temp. |
| H. influenzae | ○ | ○ | ○ | | ○ | ○ | ○ | |
| P. aeruginosa | ○ | ○ | ○ | | ○ | ○ | ○ | |
| B. cepacia | ○ | ○ | ○ | | ○ | ○ | ○ | |
| S. aureus | ○ | ○ | ○ | | ○ | ○ | ○ | |
| S. maltophilia | ○ | ○ | ○ | | ○ | ○ | ○ | |
| A. xylosoxidans | ○ | ○ | ○ | | ○ | ○ | ○ | |
| Fungi | ○ | ○ | ○ | | ○ | ○ | ○ | |

**9.** Do you perform a special test for cultivation of **anaerobic bacteria**?

○ Yes ○ No

What kind of agar do you use for anaerobic cultures?

○ Anaerobian blood agar
○ Kanamycin Vancomycin blood agar
○ Phenylethyl Alcohol agar
○ Others: _____

**10.** How is the **differentiation of pathogens** performed in your laboratory?

| **System** | **for differentiation of** |
|---|---|
| ○ API® systems | |
| ○ API Staph | |
| ○ API 20 Strep | |
| ○ RapiD 20 E | |
| ○ API 20 E | |
| ○ API 20 NE | |
| ○ VITEK® 1 | |
| ○ VITEK® 2 | |
| ○ BD Phoenix™ | |
| ○ MicroScan® | |
| ○ MALDI-TOF | |
| ○ Others: | |

**11.** Do you identify **Gram-negative nonfermenting bacteria** other than P. aeruginosa and/or B. cepacia?

○ Yes ○ No

What kind of system do you use therefore?

- ○ API 20 NE
- ○ VITEK® 1
- ○ VITEK® 2
- ○ BD Phoenix™
- ○ MicroScan®
- ○ MALDI-TOF
- ○ 16S Sequencing
- ○ Others: _____

**12.** How is the **susceptibility testing** done?

- ○ VITEK® 1
- ○ VITEK® 2
- ○ BD Phoenix™
- ○ MicroScan®
- ○ Others: _____

- ○ Quantitative determination of the MIC via
  - ○ Agar dilution
  - ○ Microdilution
  - ○ Etest®
  - ○ Others: _____
- ○ Qualitative susceptibility testing via
  - ○ Agar diffusion
  - ○ Others: _____

**13.** Do you perform **antibiotic combination tests?**

○ Yes ○ No

With what kind of system?

- ○ Merlin
- ○ Etest®
- ○ In house
- ○ Others: _____

**14.** Do you routinely investigate the **following pathogens**?

| | Yes | No | On request |
|---|---|---|---|
| **Mycobacteria** | ○ | ○ | ○ |
| **Viruses** | ○ | ○ | ○ |

**15.** Do you perform **confirmatory tests** for special pathogens?

○ Yes ○ No

What kind of confirmatory test do you perform for which pathogen?

| | PCR | 16S Sequencing | Probe | Others: |
|---|---|---|---|---|
| MRSA | ○ | ○ | ○ | |
| B. cepacia | ○ | ○ | ○ | |
| Atypical Mycobacteria | ○ | ○ | ○ | |
| Others: | ○ | ○ | ○ | |

**16.** Do you send unusual pathogens to **reference laboratories** for further differentiation and typing?

○ Yes ○ No

What kind of pathogens?
- ○ MRSA
- ○ B. cepacia
- ○ Atypical Mycobacteria
- ○ Uncommen yeasts
- ○ Uncommen fungi
- ○ Others: _____

**17.** Do you inform the CF center to initiate **special hygiene measures** if **B. cepacia** was isolated?

○ Yes ○ No

**18.** Do you inform the clinician if pathogens with **special phenotypes** were isolated?

| | Yes | No |
|---|---|---|
| **SCV** | ○ | ○ |
| **Mucoide phenotypes** | ○ | ○ |
| **Others:** | ○ | ○ |

**19. In how many CF patients** did you detect the following pathogens during the last year?

| **CF patients with** | **Number** |
|---|---|
| MRSA | |
| B. cenocepacia | |
| B. multivorans | |
| Other Burkholderia | |
| Atypical Mycobacteria | |
| M. abscessus | |
| M. avium intracellulare | |
| M. chelonae | |
| Other Mycobacteria | |
| Pandoraea species | |
| Exophiala dermatitidis | |
| Scedosporium apiospermum | |
| Other uncommon fungi | |

TABLE S1 Translation of the main recommendations by the MiQ 2006

| **Culture - Stepwise testing** | |
|---|---|
| **Step 0 - Basis culture media** | |
| No colonization with *P. aeruginosa*/other pathogens (especially nonfermenting bacteria) → no overgrowth expected | |
| Blood agar | E.g. Columbia blood agar, Schaedler agar with 5% sheep's blood |
| Chocolate agar | Supplemented with bacitracin |
| Lactose indicator agar | E.g. MacConkey agar |
| CF-specific at all steps | *Pseudomonas* agar |
| | *B. cepacia* selective agar |
| Optional | Selective medium for *S. aureus* |
| **Step 1 - Extended culture media** | |
| Colonization with *P. aeruginosa*/other nonfermenting pathogens or mixed infection → probable overgrowth of slow growing pathogens or variant pathogens | |
| Basis culture media | |
| Selective medium for *S. aureus* | E.g. mannitol salt agar, CHROMagar Staph aureus, *S. aureus* ID agar |

| | |
|---|---|
| Optional | Meropenem agar, quantitative culture, when indicated special anaerobic culture for *H. influenza*, fungi medium |

**Step 2 - Extended culture media**

Colonization/infection with common pathogens for > 1 year with repeated antibiotic therapies → probable selection of resistant variants

Basis culture media

Meropenem agar

Quantitative culture

| | |
|---|---|
| Optional | Special anaerobic culture for *H. influenza*, fungi medium |

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**Culture media for the detection of common CF pathogens** (medium of choice listed first)

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| | |
|---|---|
| *S. aureus* | • Columbia blood agar<br>• *S. aureus* ID agar<br>• Mannitol salt agar<br>• CHROMagar Staph aureus<br>• BHI-NaCl-Agar |

| | |
|---|---|
| | • Polymyxin/Nalidixic acid agar |
| | • Baird-Parker agar |
| | • MRSA selective agar |
| *H. influenza* | • Chocolate agar with bacitracin |
| | • When indicated anaerobic culture |
| *P.aeruginosa* | • Tryptic Soy Agar |
| | • MacConkey agar |
| | • Cetrimid agar |
| | • Pseudomonas Isolation Agar |
| BCC | • BCSA |
| | • OFPBL agar |
| | • PC agar |

**Identification of pathogens**

*P. aeruginosa* and other nonfermenting pathogens

Identification should be done to species level using

- a commercial method preferring API 20 NE or

- a molecular method e.g. 16S rDNA sequencing, species-specific PCR or FISH

Metabolic inactive nonfermenting pathogens e.g. lung adapted variants of *P. aeruginosa*, BCC amongst others can't be identified to species level in part using commercial systems

BCC isolates must be confirmed by a molecular method, obligatory when initially isolated

Method of choice: *recA* sequencing

Alternatives: RFLP of *recA* gene, species-specific PCR

*S. aureus*

Doubtful results should be confirmed by *S. aureus* specific PCR

Especially in case of culture of *S. aureus* SCVs, an identification at the species level is often only possible by PCR

Isolates that can't be differentiated should be determined with molecular methods e.g. 16S rDNA sequencing

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**Confirmatory tests for**

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MRSA

- Gold standard: detection of the *mecA* gene as "in-house" or commercial PCR
- Detection of PBP 2a

| | |
|---|---|
| BCC | • RFLP of the *recA* gene<br>• Species-specific PCR based on polymorphisms in the rDNA und *recA* gene<br>• *RecA* sequencing and phylogenetic analysis of *recA* gene sequences are recommended |

**Susceptibility testing**

Guided by CLSI or DIN 58940

Quantitative determination of the MIC via

- Dilution methods e.g. agar or bouillon dilution
- Gradient diffusion methods e.g. Etest

Qualitative susceptibility testing via Agar diffusion

Method of choice for all nonfermenting bacteria:

- Agar diffusion or
- Dilution methods preferring microdilution

Susceptibility testing of most *S. aureus* isolates can be done with commercial systems

Automated systems are not recommended for susceptibility testing of *P. aeru-*

*ginosa* and other nonfermenting pathogens because they are more often defective and little validated

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TABLE S2 Different BCC selective agar formulations

| Agar | Formulation |
|---|---|
| *B. cepacia* selective agar | 10 g of lactose and 10 g of sucrose in an enriched base of casein and yeast extract with 600,000 U of polymyxin, 10 mg of gentamicin and 2.5 mg of vancomycin per liter[a] |
| *B. cepacia* agar | DeCicco holding medium with 300,000 U of polymyxin and 100 mg of ticarcillin per liter[b] |
| cepacia medium | 300,000 U of polymyxin and 0.1 of ticarcillin per liter[c] |
| Oxoid *B. cepacia* agar | 150,000 IU of polymyxin, 5 mg of gentamicin and 100 mg of ticarcillin per liter[d] |
| oxidative-fermentative polymyxin B-bactriacin lactose agar | oxidation-fermentation agar supplemented with 10 g of lactose, 300,000 U of polymyxin and 200 U of bacitracin per liter[e] |

[a] Data from reference 29.

[b] Data from reference 16.

[c] Data from reference 30.

[d] Data from reference 31.

[e] Data from reference 32.