

Supplementary material

Rapid syndromic PCR testing in patients with respiratory tract infections reduces time to results and improves microbial yield

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Supplementary material section one (S1)

Supplementary table 1. BioFire® FilmArray® Pneumonia panel <i>plus</i> (FAP <i>plus</i>) targets	
<u>Bacteria (semi-quantitative)</u>	<u>Atypical bacteria (qualitative)</u>
<i>Acinetobacter calcoaceticus-baumannii</i> complex	<i>Legionella pneumophila</i>
<i>Enterobacter cloacae</i>	<i>Mycoplasma pneumoniae</i>
<i>Escherichia coli</i>	<i>Chlamydia pneumoniae</i>
<i>Haemophilus influenzae</i>	
<i>Klebsiella aerogenes</i>	<u>Viruses (qualitative)</u>
<i>Klebsiella oxytoca</i>	Influenza A virus
<i>Klebsiella pneumoniae</i> group	Influenza B virus
<i>Moraxella catarrhalis</i>	Adenovirus
<i>Proteus spp.</i>	Coronavirus
<i>Pseudomonas aeruginosa</i>	Parainfluenza virus 1-4
<i>Serratia marcescens</i>	Respiratory syncytial virus
<i>Staphylococcus aureus</i>	Human rhinovirus/enterovirus
<i>Streptococcus agalactiae</i>	Human metapneumovirus
<i>Streptococcus pneumoniae</i>	Middle East respiratory syndrome coronavirus
<i>Streptococcus pyogenes</i>	
<u>Antibiotic resistance genes</u>	
ESBL:	
- CTX-M	
Carbapenemases:	
- KPC	
- NDM	
- OXA-48-like	
- VIM	
- IMP	
Methicilin Resistance:	
- mecA/mecC and MREJ	

Supplementary material section two (S2)

Inclusion- and exclusion criteria

Inclusion criteria:

- Adult aged ≥ 18 years
- Admitted to the emergency department (ED) with suspicion of community acquired pneumonia (CAP)
- Fulfilling at least two of the following criteria: new or worsening cough; new or worsening expectoration of sputum; new or worsening dyspnea; hemoptysis; pleuritic chest pain; radiological evidence of CAP; abnormalities on chest auscultation and/or percussion; fever (≥ 38.0 °C).

Exclusion criteria:

- Hospitalization (>48 hours) within the last 14 days prior to admission
- Cystic fibrosis
- Severe bronchiectasis
- A palliative approach (defined as life expectancy below two weeks)
- Patient not willing or able to provide a lower respiratory tract sample

Definitions and diagnostic clinical criteria for CAP. Adapted from Postma D.F. et al. ¹

Clinical CAP:

- Patients with the presence of at least two diagnostic criteria* and in-hospital treatment and/or diagnosis of clinically suspected CAP documented by the treating physician and in agreement with the assessment by a study investigator. In case of disagreement, an additional study investigator will arbitrate.
- Patients with two or more diagnostic criteria and an obvious non-respiratory source of infection are not considered to have a clinical diagnosis of CAP.

Radiologically confirmed CAP:

- Clinical CAP
- The presence of a new or increased infiltrate on chest radiography or computer tomography (CT).

* Diagnostic criteria:

- Cough: recent or worsening
- Production of purulent sputum or a change in the character of sputum
- Temperature >38.0 °C or <36.1 °C
- Auscultatory findings consistent with pneumonia, including rales, evidence of pulmonary consolidation (dullness on percussion, bronchial breath sounds, or egophony), or both
- Leukocytosis ($>11.0 \times 10^9$ white cells per liter or $> 8.2 \times 10^9$ neutrophils)
- C-reactive protein (CRP) level > 50 mg/L
- Dyspnea, tachypnea, or hypoxemia: recent or worsening

Supplementary material section three (S3) ²⁻⁵

Sputum induction by isotonic and/or hypertonic saline

Contraindications

Known airway obstruction due to high humidity or previous serious side effects due to inhalation with saline

Isotonic NaCl (0.9%)

- Patients with spontaneous production and expectoration of sputum
- Always the primary choice in patients with asthma, severe chronic obstructive pulmonary disease (COPD), obstructive airways (prolonged expirium, wheezing sounds), hypoxemia (SpO₂ < 92 %) or other known condition with hyper reactive airways
- Contact a physician for evaluation if:
 - Severe COPD (GOLD 3-4) with history of CO₂-retention
 - If salbutamol (Ventoline)/ipratropiumbromid (Atrovent) is considered, a physician should be contacted if the patient is:
 - Pregnant
 - Has tachycardia/atrial fibrillation (persistent pulse > 120)

Hypertonic NaCl (1mmol/ml - 5.8 %)

- Patients with little sputum production and little cough
- Contact a physician for evaluation if:
 - Pregnant (**shall not be given to pregnant women**)
 - Obstructive airways (prolonged expirium, wheezing sounds)
 - Known asthma, severe COPD (GOLD 3-4) or other known condition with hyper reactive airways
 - SpO₂ < 92 %
 - Tachycardia/atrial fibrillation (persistent pulse > 120)

Implementation

Use hand disinfection and gloves when handling medications and equipment

- Use droplet precautions
- Only use sterile saline in the nebulizer and check the expiration date
- 5 ml NaCl (either isotonic or hypertonic) is put in the nebulization chamber

- If known asthma, severe COPD (GOLD 3-4), obstructive airways (prolonged expiration, wheezing sounds) or other known condition with hyper reactive airways:
Use 2.5 ml NaCl and 2.5 ml salbutamol (Ventoline) (2 mg/ml)
- Mouthpiece is the first choice, but masks can also be used. Use nose clip if mouthpiece is used
- Adjust the airflow to 8 liters/minute. Check that the nebulizer is working and producing fine mist
- Observe the patients with regard to worsened respiratory symptoms. If this happens, abort the procedure, contact a physician and start salbutamol (5 mg) and ipratropiumbromid (0.5 mg) in the nebulizer
- If the patients do not provide sputum after the first round of nebulizing, and no side effects are reported, the preceding steps can be repeated by using hypertonic NaCl 1 mmol/ml (5.8%). If hypertonic NaCl was given without any bronchodilator in the first round, please add salbutamol (Ventoline) in the second round
- Avoid spills or soiling the outside of the container
- Inspect the volume, color and consistence of the material to decide if the test is of good quality
- Clean/disinfect contact points after the procedure

Procedure diagnostic endotracheal aspiration

Equipment

- Face mask with visor/glasses
- Clean gloves
- Wall mounted suction with hose
- Suction catheter with bent tip 14 Ch (green)
- Tracheal mucus extractor
- Lidocain gel
- Sterile container
- 10 mL sterile isotonic saline

Procedure

- The patient should be positioned upright with the head slightly tilted backwards and the chin forwards
- Test the suction
- Connect the tracheal mucus extractor
- Use lidocaine gel at the tip of the catheter and in the patient's nose
- Do not touch the distal 15 cm of the catheter
- The catheter is inserted through the nose and down to the hypopharynx without suction, until respiratory sounds are audible through the catheter end
- The patient is then instructed to cough or take a deep breath, while the catheter is lead 3-5 cm further down into the trachea
- The catheter is then connected to the tracheal mucus extractor
- Start suction, but no more than 15 seconds at a time and without moving the catheter
- The catheter is retracted slowly with a rotating movement, without suction
- After the procedure, if necessary, the catheter can be flushed with 1-2 mL sterile isotonic saline to collect any remaining respiratory specimens into the sample container

Supplementary material section four (S4)

Determining the clinical relevance of microbiological detections⁶⁻²³

1. Clinically relevant

A. Proven etiology

Diagnosis of CAP AND

- i. Detection of the same bacteria in the respiratory tract specimen and blood culture
- ii. *Streptococcus pneumoniae* detected in respiratory tract specimen and positive pneumococcal urine antigen test
- iii. Detection of *C. pneumoniae*, *B. pertussis*, *L. pneumophila* or *M. pneumoniae* in respiratory tract specimen
- iv. Positive urine antigen test for *L. pneumophila*
- v. Growth of category B- or C-bacteria (Supplementary table 2) in blood culture and no other detectable focus of infection

B. Probable etiology

Diagnosis of CAP AND

- i. Detection of category B-bacteria in a respiratory tract specimen
- ii. Detection of category C-bacteria in respiratory tract specimens in a patient with chronic underlying disease^a and/or patients with a recent history of antibiotic use^b
- iii. Detection of a single species of category D-bacteria in a respiratory tract specimen in immunocompromised patients^c and/or patients with a recent history of antibiotic use or other known risk of infections due to certain bacteria^d
- iv. Detection of category D-bacteria in blood culture and no other detectable focus of infection
- v. Positive pneumococcal urine antigen test

2. Uncertain relevance

- i. All detections not defined by the above criteria.

^a Such as chronic lung disease, diabetes mellitus, chronic kidney disease, immunosuppression

^b Two courses of antibiotics in the last 6 months

^c Chronic lung disease, except for asthma, acquired or congenital immunodeficiency, including HIV-infection, chronic use of corticosteroids > 7,5 mg prednisolone or equipotent dosage, use of immunosuppressants (ATC L04), ongoing treatment with cytostatic (ATC L01).

^d >Prior infection with resistant microbes (e.g., extended spectrum beta-lactamase (ESBL), Methicillin-resistant *Staphylococcus aureus* (MRSA)).

Supplementary table 2. Respiratory tract microbes divided in different categories according to their potential clinical relevance	
Category	Microbe
<p>A: Always pathogens Always considered relevant in a patient with respiratory tract infection</p>	<p>Adenovirus^{9,11,15,17} Coronavirus^{11,15,17} Human metapneumovirus^{9,15,17} Influenza virus^{9,11,15,17} MERS¹² Parainfluenza virus^{11,15,17} Rhinovirus^{9,15,17} RS-virus^{9,11,15,17}</p> <p><i>Bordetella pertussis</i>⁹ <i>Chlamydia pneumoniae</i>^{6,9,11,15,17} <i>Legionella pneumophila</i>^{6,9,17} <i>Mycoplasma pneumoniae</i>^{6,9,11,15,17}</p>
<p>B: Usually pathogens Can be colonizers, but are usually considered relevant in a patient with pneumonia</p>	<p><i>Haemophilus influenzae</i>^{6,9,11,15,17} <i>Streptococcus pneumoniae</i>^{6,9,11,15,17,23} <i>Streptococcus pyogenes</i>^{14,21,23}</p>
<p>C: Usually not pathogens Usually colonizers, but can cause pneumonia, especially in patient with chronic diseases</p>	<p><i>Klebsiella pneumoniae</i>^{10,17,23} <i>Moraxella catarrhalis</i>^{6,8,15,17} <i>Staphylococcus aureus</i>^{6,15,17,18,23}</p>
<p>D: Usually not pathogens A seldom cause of pneumonia in otherwise healthy patients. May be considered relevant if detected as the only bacterial species in a patient with immunosuppression and/or several previous courses of antibiotics</p>	<p><i>Acinetobacter calcoaceticus-baumannii-complex</i>^{7,15,23} <i>Enterobacter cloacae</i>^{9,15,22} <i>Escherichia coli</i>^{9,15,17,22,23} <i>Klebsiella aerogenes</i>^{9,15} <i>Klebsiella oxytoca</i>^{9,15} <i>Proteus species</i>^{9,15,22} <i>Pseudomonas aeruginosa</i>^{15-17,22,23} <i>Serratia marcescens</i>^{9,15,22} <i>Streptococcus agalactiae</i>¹⁹</p>

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