Bacterial Lung Infection

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Bacterial lung infection: Pneumonia and Tuberculosis

- Pneumonia: inflammation of the lung parenchyma
  - consolidation of the affected part: a filling of the alveolar air spaces with exudate, inflammatory cells, and fibrin
  - The most common cause is infection by bacteria or viruses
  - Other causes: infection by other infectious agents such as rickettsiae, fungi, and yeasts
Classification and Categorization

• Based on various characteristics of the illness
  – anatomic or radiologic distribution
  – the setting of infection
    • community
    • institutional (healthcare/nursing home setting)
    • nosocomial (hospital)
  – the mechanism of acquisition
    • ventilator use
    • aspiration
  – the pathogen responsible
Anatomic or radiologic distribution

• Lobar - known as focal or nonsegmental pneumonia
  – Confluent consolidation involving a complete lung lobe
  – Most often due to *Streptococcus pneumoniae* (pneumococcus)
  – Can be seen with other organisms (*Klebsiella, Legionella*)

• Multifocal/lobular (bronchopneumonia)
  – Infection starting in airways and spreading to adjacent alveolar lung
  – Most often seen in the context of pre-existing disease

• Interstitial (focal or diffuse)
Lobar pneumonia

Bilateral lower lobe pneumonia
The setting or mechanism of infection

• Community-acquired pneumonia (CAP)
  – develops in the outpatient setting or within 48 hours of admission to a hospital
  – should not meet the criteria for healthcare-associated pneumonia
The setting or mechanism of infection

- Healthcare-associated/nursing home-associated pneumonia (HCAP/NHAP): develops in the outpatient setting or within 48 hours of admission in patients with increased risk of exposure to MDR bacteria as a cause of infection
  - Hospitalization for ≥ 2 days in an acute care facility ≤ 90 days of current illness
  - Exposure to antibiotics, chemotherapy, or wound care ≤ 30 days of current illness
  - Residence in a nursing home or long-term care facility
  - Hemodialysis at a hospital or clinic
  - Home nursing care (infusion therapy, wound care)
  - Contact with a family member or other close person with infection due to MDR bacteria

MDR = multidrug-resistant
The setting or mechanism of infection

- Nosocomial pneumonia: hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP)
  - HAP: develops > 48 hours after admission to a hospital
  - VAP: develops > 48 hours after endotracheal intubation or ≤ 48 hours of extubation
- Aspiration pneumonia: develops after the inhalation of oropharyngeal secretions and colonized organisms
Pathophysiology

• Extrinsic causes
  – exposure to a causative agent
  – require enough pathogens to overwhelm resident defenses

• Intrinsic causes
  – loss of protective upper airway reflexes, e.g., altered mental status due to intoxication and other metabolic states; neurologic causes, such as stroke; endotracheal intubation
  – an impairment of the immune response, e.g., HIV infection, chronic disease, advanced age
  – dysfunction of defense mechanisms, e.g., smoking, COPD, tumors, inhaled toxins, aspiration
  – poor dentition or chronic periodontitis
Bacteria from upper airways or, less commonly, from hematogenous spread

Virulence of the infecting organism, status of the local defenses, overall health of the patient, etc.

Bacterial pneumonia
Pathophysiology

• Bacterial virulence
  – Development of resistance to various classes of antibiotics
  – Flagellae and other bacterial appendages that facilitate spread of infection
  – Capsules resistant to attack by immune defense cells and that facilitate adhesion to host cells
  – Quorum sensing systems allow coordination of gene expression based on complex cell-signaling for adaptation to the local cellular environment
  – Iron scavenging
Pathophysiology

• Host resistance
  – Deficits in various host defenses and an inability to mount an appropriate acute inflammatory response
    • Deficits in neutrophil quantity, as in neutropenia
    • Deficits in neutrophil quality, as in chronic granulomatous disease
    • Deficiencies of complement
    • Deficiencies of immunoglobulins
Pathophysiology

• Viral infection can have an important role in bacterial pneumonia
  – alteration of pulmonary physiology
  – downregulation of the host immune defense
  – changes in expression of receptors to which bacteria adhere
  – enhancement of the inflammatory process
Etiology

• Typical organisms
  – Gram-positive bacteria: *Streptococcus pneumoniae* (the most common cause), *Staphylococcus aureus* (intravenous drug abusers, debilitating persons), *Enterococcus* (*E. faecalis*, *E. faecium*), *Actinomyces israelii* (known to form abscesses and sulfur granules), *Nocardia asteroides* (cause lung abscesses and cavitations)
  – Gram-negative bacteria: *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* (chronic alcoholism, diabetes, or COPD), *Haemophilus influenzae*, *Moraxella catarrhalis*, *Acinetobacter baumannii* (ventilator-associated pneumonia)
Etiology

• Atypical organisms: *Mycoplasma* species, *Chlamydophila* species (*C. psittaci*, *C. pneumoniae*), *Legionella* species (the causative agents of Legionnaires disease),
  – generally associated with a milder form of pneumonia (walking pneumonia)
  – unable to detect them on Gram stain or to cultivate them in standard bacteriologic media
Etiology

• Anaerobic organisms
  – typically results from aspiration of oropharyngeal contents
  – tend to be polymicrobial
  – may consist of the following anaerobic species: *Klebsiella, Peptostreptococcus, Bacteroides, Fusobacterium, Prevotella*
# Etiology of community-acquired pneumonia

<table>
<thead>
<tr>
<th>Outpatient CAP</th>
<th>Inpatient CAP</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td><em>Streptococcus pneumoniae</em></td>
</tr>
<tr>
<td>Atypical pathogens <em>(Chlamydophila pneumoniae, Mycoplasma pneumoniae, Legionella spp.)</em></td>
<td>Atypical pathogens <em>(Chlamydophila pneumoniae, Mycoplasma pneumoniae)</em></td>
</tr>
<tr>
<td>Viruses</td>
<td><em>Staphylococcus aureus</em>, Gram negative enteric bacteria</td>
</tr>
</tbody>
</table>

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*Outpatient CAP:* The common etiology of outpatient CAP includes *Streptococcus pneumoniae*. Atypical pathogens such as *Chlamydophila pneumoniae*, *Mycoplasma pneumoniae*, and *Legionella spp.* can also be responsible. Viruses are less common causes.

*Inpatient CAP:* Inpatient CAP often has a more severe presentation and can be caused by *Streptococcus pneumoniae*. Atypical pathogens, similar to outpatient CAP, can also be involved. *Staphylococcus aureus* and Gram negative enteric bacteria are additional etiologies for inpatient CAP.
History

• Potential exposures
  – contaminated air-conditioning or water systems – *Legionella* species
  – overcrowded institutions (e.g., jails, homeless shelters) – *S. pneumoniae, Mycobacteria, Mycoplasma, Chlamydophila*
  – various types of animals - cats, cattle, sheep, goats (*Coxiella burnetii, Bacillus anthracis* [cattle hide]); turkeys, chickens, ducks, or other birds (*C. psittaci*); rabbits, rodents (*Francisella tularensis, Yersinia pestis*)
History

Aspiration risks

- Alcoholism
- Altered mental status
- Anatomic abnormalities, congenital or acquired
- Dysphagia
- Gastroesophageal reflux disease (GERD)
- Seizure disorder

Host factors

- Comorbid conditions (e.g., asthma, COPD, smoking, and a compromised immune system are risk factors for *H. influenzae* infection)
- Previous surgeries
- Possibility of immunosuppression
- Social and sexual history
- Family history
- Medication history
- Allergy history
Symptoms

Sudden onset/rapid illness progression
- cough (particularly productive cough)
- chest pain
- Dyspnea
- Hemoptysis
- decreased exercise tolerance
- abdominal pain from pleuritis

The character of the sputum
- *S. pneumoniae*: rust-colored sputum
- *Pseudomonas, Haemophilus*, and pneumococcal species: green sputum
- *Klebsiella* species: red currant-jelly sputum
- Anaerobic infections: foul-smelling or bad-tasting sputum
Nonspecific Symptoms

• Common: fever, rigors or shaking chills, malaise
• Other nonspecific symptoms: myalgias, headache, abdominal pain, nausea, vomiting, diarrhea, anorexia, weight loss, altered sensorium
• *Legionella* pneumonia
  – Often present with mental status changes or diarrhea
  – Patients may develop hemoptysis or pulmonary cavitations
  – >50% of *Legionella* pneumonia: GI symptoms such as anorexia, nausea, vomiting, and diarrhea
Physical Examination

Depends on type of organism, severity of infection, coexisting host factors, and the presence of complications

• Signs
  – Hyperthermia (fever, typically > 38°C) or hypothermia (< 35°C)
  – Tachypnea (> 18 respirations/min)
  – Use of accessory respiratory muscles
  – Tachycardia (> 100 bpm) or bradycardia (< 60 bpm)
  – Central cyanosis
  – Altered mental status

• Physical findings
  – Adventitious breath sounds, such as rales/crackles, rhonchi, or wheezes
  – Decreased intensity of breath sounds
  – Egophony
  – Whispering pectoriloquy
  – Dullness to percussion
  – Tracheal deviation
  – Lymphadenopathy
  – Pleural friction rub
Physical Examination

• Examination findings that may indicate a specific etiology
  – Bradycardia: *Legionella* infection
  – Periodontal disease: anaerobic and/or polymicrobial infection
  – Bullous myringitis: *Mycoplasma pneumoniae* infection
  – Physical evidence of risk for aspiration: a decreased gag reflex
  – Cutaneous nodules, especially in the setting of central nervous system (CNS) findings: *Nocardia* infection
Workup

• Patients with CAP should be investigated for specific pathogens that would significantly alter standard (empirical) management decisions

• Imaging studies are generally helpful in
  – detecting suspected pneumonia
  – identifying the presence of complications
  – suggesting specific pathogens occasionally
Workup

• Blood studies
  – CBC count with differential: leukocytosis with a left shift; leukopenia may be an ominous clinical sign of impending sepsis
  – Blood cultures: show poor sensitivity in pneumonia (positive in ~40% of cases)
  – rarely dictates a change in antibiotic use
Workup

- **Sputum Gram stain and culture**
  - should be performed before initiating antibiotic therapy
  - adequate sputum contains neutrophils > 25 cells/low-power field (LPF) and squamous epithelial cells < 10 cells/LPF on microscopic examination
  - a single predominant microbe should be noted
  - mixed flora may be observed with anaerobic infections
  - only specimen that have satisfied the criteria above should be submitted for culturing
Workup

- Sputum Gram stain and culture
  - Normal respiratory flora

<table>
<thead>
<tr>
<th>Site</th>
<th>Common or medically important organisms</th>
<th>Less common but notable organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nose</td>
<td><em>Staphylococcus aureus</em></td>
<td><em>S. epidermidis</em>, diphtheroids, assorted streptococci</td>
</tr>
<tr>
<td>Oropharynx</td>
<td>Viridans streptococci, including <em>S. mutans</em></td>
<td>Assorted streptococci, nonpathogenic <em>Neisseria</em> (e.g., <em>N. mucosa</em>), nontypeable <em>Haemophilus influenzae</em></td>
</tr>
<tr>
<td>Gingival crevices</td>
<td>Anaerobes: <em>Bacteroides</em>, <em>Prevotella</em>, <em>Fusobacterium</em>, <em>Streptococcus</em>, <em>Actinomyces</em></td>
<td>-</td>
</tr>
</tbody>
</table>

- The presence of normal flora does not rule out infection
Quantitative Reporting Values

<table>
<thead>
<tr>
<th>Report as</th>
<th>No.</th>
<th>Epithelial cells/PMNs (cells/LPF)</th>
<th>Microorganisms (cells/oil field)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rare</td>
<td>≤ 1</td>
<td></td>
<td>≤ 1</td>
</tr>
<tr>
<td>Few</td>
<td>1-9</td>
<td></td>
<td>1-5</td>
</tr>
<tr>
<td>Moderate</td>
<td>10-25</td>
<td></td>
<td>6-30</td>
</tr>
<tr>
<td>Many</td>
<td>&gt; 25</td>
<td></td>
<td>&gt; 30</td>
</tr>
</tbody>
</table>

PMNs – polymorphonuclear cells
LPF – low power field (x10 objective lens)
Oil field – x100 objective lens
*Streptococcus pneumoniae* is the most common cause of bacterial pneumonia. Typically, the organisms are arranged in pairs and are lancet shaped. They are frequently surrounded by a large capsule but it is usually difficult to see this on a Gram stained smear. The capsule is seen as a halo surrounding the cells. These organisms should be reported as lancet shaped Gram positive cocci in pairs.

*Courtesy of Hilaire Thomas*
Staphylococci are usually much larger than Streptococci. They are usually round or slightly oval cocci which occur singly, in pairs or in small clusters. Report these organisms as Gram positive cocci in pairs and clusters.

Courtesy of Hilaire Thomas
The presence of many very tiny pleomorphic Gram negative rods is strongly suggestive of *Haemophilus influenzae*. They should be reported as small, pleomorphic Gram negative rods.
*Haemophilus influenzae* often stains very weakly and tends to blend into the background material in the smear and it is easy to overlook them. On this slide, they are most clearly seen as intracellular organisms in the poly with a 4-lobed nucleus. Look for more amongst the background material.

Courtesy of Hilaire Thomas
*Moraxella catarrhalis.* A large number of Gram negative cocci are seen and many appear to be attaching to or residing within the PMNs.
Acinetobacter spp.: Gram negative coccobacilli or kidney-shaped diplococci resemble *Neisseria* spp. or *Moraxella* spp. depends on clinical setting.
Enterobacteriaceae

These organisms are gram negative rods but lack any more specific differential characteristics. They are usually short fat rods, larger than Haemophilus sp.
Pseudomonas sp.

Pseudomonads are usually long slender Gram negative rods. Like Enterobacteriaceae, their morphology is not sufficiently typical to be able to characterize them on a Gram smear.

Courtesy of Hilaire Thomas
This slide represents mixture of *Haemophilus* and pneumococci.

Courtesy of Hilaire Thomas
When multiple morphologic types are present they are reported as “Mixed Organisms”. This slide shows heavy contamination with oropharyngeal flora including Yeasts.

Courtesy of Hilaire Thomas
You will recognize these as probable staphylococci.

Courtesy of Hilaire Thomas
Workup

- Chest Radiography
  - Considered to be the criterion standard for diagnosing pneumonia
  - the presence of an infiltrate is required for the diagnosis
  - the accuracy of plain chest radiography decreases depending on the setting of infection
  - pleural effusion is present in ~ half of individuals with *H. influenzae* pneumonia
Recommended Diagnostic Testing in Patients with Suspected Community-Acquired Pneumonia

<table>
<thead>
<tr>
<th>Indication</th>
<th>Blood culture</th>
<th>Sputum culture</th>
<th>Legionella UAT</th>
<th>Pneumococcal UAT</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intensive care unit admission</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X^a</td>
</tr>
<tr>
<td>Failure of outpatient antibiotic therapy</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td>X^b</td>
</tr>
<tr>
<td>Cavitary infiltrates</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukopenia</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active alcohol abuse</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
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<tr>
<td>Chronic severe liver disease</td>
<td>X</td>
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<tr>
<td>Severe obstructive/structural lung disease</td>
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<tr>
<td>Asplenia (anatomic or functional)</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Recent travel (within past 2 weeks)</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td>X^c</td>
</tr>
<tr>
<td>Positive Legionella UAT result</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>X^d</td>
</tr>
<tr>
<td>Positive pneumococcal UAT result</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>Pleural effusion</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X^e</td>
</tr>
</tbody>
</table>

**NOTE.** NA, not applicable; UAT, urinary antigen test.

^a Endotracheal aspirate if intubated, possibly bronchoscopy or nonbronchoscopic bronchoalveolar lavage.

^b Fungal and tuberculosis cultures.

^c Special media for Legionella.

^d Thoracentesis and pleural fluid cultures.

Hotel or cruise ship stay in previous 2 weeks - *Legionella* species

Travel to or residence in Southeast and East Asia - *Burkholderia pseudomallei*, avian influenza, SARS (severe acute respiratory syndrome)
<table>
<thead>
<tr>
<th>Etiologic Agents</th>
<th>Diagnostic Procedures</th>
<th>Optimum Specimens</th>
<th>Transport Issues; Optimal Transport Time</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>Gram stain, Culture, Urine antigen</td>
<td>Sputum, bronchoscopic specimens, Urine</td>
<td>Sterile container, room temperature (RT), 2 h; &gt;2–24 h, 4°C; Sterile container, RT, 24 h; &gt;24 h–14 d, 2–8°C</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Gram stain, Culture</td>
<td>Sputum, bronchoscopic specimens</td>
<td>Sterile container, RT, 2 h; &gt;2–24 h, 4°C</td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em></td>
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<tr>
<td><em>Enterobacteriaceae</em></td>
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<tr>
<td><em>Pseudomonas aeruginosa</em></td>
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<tr>
<td><em>Legionella species</em></td>
<td>Urine antigen, <em>L. pneumophila</em> serogroup 1, Selective culture on buffered charcoal yeast extract (BCYE), Nucleic acid amplification test (NAAT)</td>
<td>Urine, Induced sputum, bronchoscopic specimens</td>
<td>Sterile container, RT, 24 h; &gt;24 h–14 d, 2–8°C; Sterile container, RT, 2 h; &gt;2–24 h, 4°C</td>
</tr>
<tr>
<td><em>Mycoplasma pneumoniae</em></td>
<td>NAAT, Serology IgM, IgG antibody detection</td>
<td>Throat swab, NP swab, sputum, bronchoalveolar lavage (BAL)</td>
<td>Transport in M4 media or other Mycoplasma-specific medium at RT or 4°C up to 48 h; ≥48 h, −70°C; Clot tube, RT, 24 h; &gt;24 h, 4°C</td>
</tr>
<tr>
<td>Etiologic Agents</td>
<td>Diagnostic Procedures</td>
<td>Optimum Specimens</td>
<td>Transport Issues; Optimal Transport Time</td>
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<tr>
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<tr>
<td><em>Chlamydia pneumoniae</em></td>
<td>NAAT</td>
<td>Nasopharyngeal (NP) swab, throat washings, sputum, bronchial specimens Serum</td>
<td>Transport in M4 or other specialized medium at RT or 4°C up to 48 h; ≥48 h, −70°C Clot tube, RT, 24 h; &gt;24 h, 4°C</td>
</tr>
<tr>
<td></td>
<td>Serology IgM antibody titer; IgG on paired serum 2–3 wk apart</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed anaerobic bacteria (Aspiration pneumonia)</td>
<td>Gram stain Aerobic and anaerobic culture</td>
<td>Bronchoscopy with protected specimen brush Pleural fluid (if available)</td>
<td>Sterile tube with 1 mL of saline or thioglycolate; RT, 2 h; &gt;2–24 h Sterile container RT, without transport ≤60 min; Anaerobic transport vial RT, 72 h</td>
</tr>
<tr>
<td>Mycobacteria</td>
<td>AFB smear AFB culture NAAT</td>
<td>Expectorated sputum; induced sputum; bronchoscopically obtained specimens</td>
<td>Sterile container, RT, ≤2 h; ≤24 h, 4°C</td>
</tr>
</tbody>
</table>

*Mycobacterium tuberculosis* and Nontuberculous Mycobacteria
Tuberculosis

• 22 million active cases in the world
• 1.7 million deaths each year (most common fatal organism)
• Incidence has increased with HIV pandemic
• Caused by *Mycobacterium tuberculosis*
  – Rod-shaped bacillus
  – Acid-fast stain
  – Nonspore forming
  – Produces mycolic acid
    • Makes it difficult to Gram stain
    • Protects the pathogen from antibiotic therapy and host defenses
Tuberculosis

- Initial symptoms are similar to those seen in other respiratory infections
  - cough (especially if lasting for 3 weeks or longer) with or without sputum production
  - coughing up blood (hemoptysis)
  - chest pain
  - loss of appetite
  - unexplained weight loss
  - night sweats
  - fever
  - fatigue
Exposure of individuals to droplet nuclei from a source case of open TB

- Duration and intensity of exposure, Immunologic defenses

  Infection

  - Weak protective immune response
    - Uncontrolled bacterial growth (primary progressive TB)

  - Strong protective immune response
    - Limited initial bacterial growth

  No infection
Host factors, bacterial factors

- Bacterial growth arrested, some bacilli persist (latent infection)
  - Immune response compromised
    - Reactivation of latent infection (reactivation TB)
  - Immune response persists, waning of dormant bacilli
    - Clearance of latent infection
- Bacterial growth arrested, all bacilli are eliminated (sterilizing immunity)
Workup

• The chest radiograph is useful for diagnosis of pulmonary TB

• Diagnostic procedures
  – AFB smear:
    • Carbolfuchsin methods: the Ziehl-Neelsen and Kinyoun methods (direct microscopy)
    • Fluorochrome procedure using auramine-O or auramine-rhodamine dyes (fluorescent microscopy)
  – AFB culture
  – NAAT
Specimen Collection Methods for Pulmonary TB Disease

- Coughing
- Induced sputum
- Bronchoscopy
- Gastric aspiration
เทคนิคการเก็บเสมหะ

ขั้นตอนที่ 1
เตรียมอุปกรณ์และตามคำแนะนำในงานที่เก็บ
เครื่องช่วยรักษาความสะอาดและเลือกสารเคมี

ขั้นตอนที่ 2
เตรียมอุปกรณ์และตามคำแนะนำในงานที่เก็บ
เครื่องช่วยรักษาความสะอาดและเลือกสารเคมี

ขั้นตอนที่ 3
ควบคุมสิ่งผักและ
เพิ่มสารเคมีกับเสมหะ
ให้คุณภาพและระบายดี

ขั้นตอนที่ 4
สุขภาพของผู้ที่ไม่ได้รับสารเคมี
คุณภาพคุณภาพให้คุณภาพ

ขั้นตอนที่ 5
ใส่กระดาษซับ

ขั้นตอนที่ 6
เน้นหัวใจความสะอาด
ต้องมีการใช้กระดาษซับ
ชัดเจน

ขั้นตอนที่ 7
หากผู้ป่วยติดเชื้อหรือมีปัญหา
คงไว้เต็มที่

ขั้นตอนที่ 8
ปิดฝากระดาษ

ขั้นตอนที่ 9
ล้างมือด้วยน้ำ
และสารทำความสะอาด
ไม่ให้เกิดอันตราย

หมายเหตุ
- ผู้ป่วยที่ไม่มีอาการหรือไม่ต้องการระบาย ควรปรับปฏิบัติเป็น
 1. ให้ผู้ป่วยเดินน้ำหนักๆ ออกกำลังกาย
 2. ให้ผู้ป่วยอดอาหาร
 3. ให้ผู้ป่วยใส่ยั้งภูมิปรับตัว
 4. ให้ผู้ป่วยบายที่ให้ยั้งภูมิปรับตัวหายจะได้
      ไม่ทำให้เกิดภาวะเสียหาย

สำนักงานโรคภูมิแพ้
กรุงเทพมหานคร
การตรวจหา acid-fast bacilli จากเสมหะ

- กลุ่มเชื้อ Mycobacteria มีผนังเซลล์ที่มีไขมันในปริมาณสูง → ติดสีย้อมทำความสะอาดได้ยาก
- สีบางชนิดที่มี phenol ผสมอยู่ด้วย เช่น carbol fuchsin จะเข้าสู่ผนังเซลล์ได้ง่ายขึ้น
- สีที่เข้าไปจะทำปฏิกิริยากับ mycolic acid ซึ่งส่วนประกอบหนึ่งของผนังเซลล์
- สารประกอบเชิงซ้อนที่เกิดขึ้นจะคงตัว ถูกชะล้างด้วย acid alcohol (3% HCl ใน 95% ethanol) ได้ง่าย
การรายงานผลการตรวจหา acid-fast bacilli (WHO)

<table>
<thead>
<tr>
<th>จำนวนเชื้อที่พบ</th>
<th>จำนวน field ที่ตรวจหาเชื้อ</th>
<th>การรายงานผล</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;10 AFB/OIF</td>
<td>20</td>
<td>Positive 3+</td>
</tr>
<tr>
<td>1-10 AFB/OIF</td>
<td>50</td>
<td>Positive 2+</td>
</tr>
<tr>
<td>10-99 AFB in 100 OIFs</td>
<td>100</td>
<td>Positive 1+</td>
</tr>
<tr>
<td>1-9 AFB in 100 OIFs</td>
<td>100</td>
<td>Scanty</td>
</tr>
<tr>
<td>No AFB in 100 OIFs</td>
<td>100</td>
<td>Negative 0</td>
</tr>
</tbody>
</table>

OIF(s) – oil immersion field(s)
Specimen Culture and Identification

• Positive cultures for *M. tuberculosis* confirm the diagnosis of TB disease.
• However in the absence of a positive culture, TB disease may be diagnosed on the basis of clinical signs and symptoms alone.
• Culture examinations should be done on all diagnostic specimens.
• The commercially available broth culture systems (e.g., BACTEC) allow detection of most mycobacterial growth in 4 to 14 days compared to 3 to 6 weeks for solid media.
Detection of *M. tuberculosis* Using Nucleic Acid Amplification Test (NAAT)

- NAAT amplifies DNA and RNA segments to rapidly identify the microorganisms in a specimen
- A single negative NAAT result should not be used as a definitive result to exclude TB disease
- Culture remains the gold standard for laboratory confirmation of TB disease