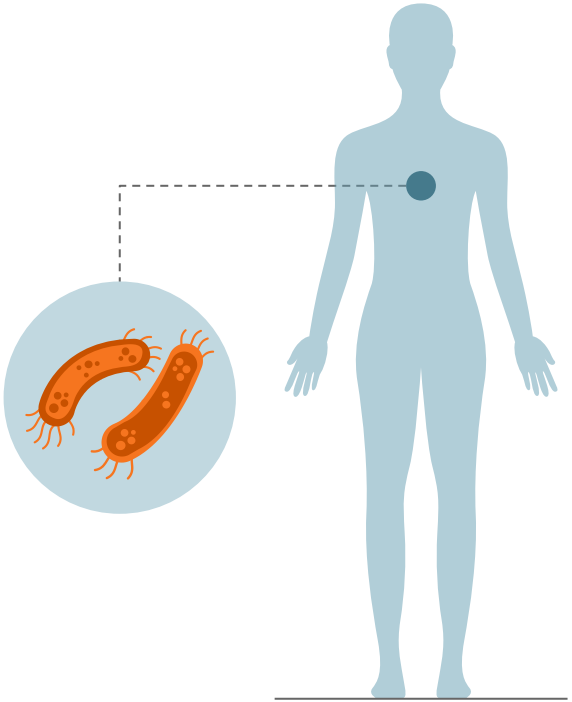

Legionella Microbiology Laboratory

Sue Lu | PATH 417A 2022W | Case 3

Case Study

To celebrate Tom's retirement his wife and two adult children accompany him on a long anticipated cruise. Tom's asthma flares up a few days before the cruise but with a corticosteroid nebulizer he feels well enough to join the cruise. Even more than the rest of his family, Tom enjoys the various hot tubs aboard the massive ship those first few days, relishing the relaxation after a busy final year at work. On the fifth day of the cruise, Tom wakes up in a sweat with a cough that continues throughout the day. As the day wears on he feels worse with a headache, muscle aches and nausea accompanying the cough. His wife arranges for the cruise doctor to visit him in his cabin. The doctor examines Tom, notes his high temperature, nonproductive cough and recent history of asthma and corticosteroid therapy. She takes a full history including taking note of his activities during the first days of the cruise and diagnoses Tom with pneumonia. She starts Tom on azithromycin. By the time the ship returns to port two days later, 5 more people have been diagnosed with a similar pneumonia, several of whom have a slightly compromised immune system, as Tom does. One of the others is admitted to hospital, where sputum and urine samples are tested and reveal a diagnosis of **Legionellosis**. Public health authorities are notified and the ship takes extra time in port to allow for an enhanced cleaning to be performed on all of the hot tubs.

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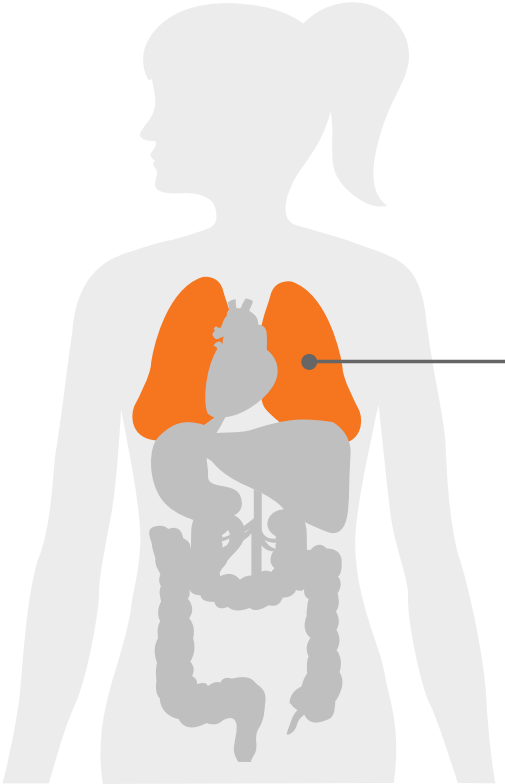
Common Pathogens that Cause Pneumonia

Common causes are generally bacterial (subcategories typical and atypical pneumonia) or viral



Bacterial

1. *Streptococcus pneumoniae* (1)
2. *Haemophilus influenzae* (1)
3. *Legionella* (atypical pneumonia) (2)
4. *Mycoplasma pneumoniae* (atypical pneumonia) (2)

Viral

1. Influenza virus (3)
2. Respiratory Syncytial Virus (RSV) (3)
3. Adenoviruses (4)

Pneumonia Diagnosis

Pneumonia diagnosis starts with a **physical examination** (5). Respiratory rate may increase, and crackles/rales may be heard (5). However, physical examination is usually not enough to make a diagnosis (5).

Pneumonia is generally diagnosed using **radiological** methods, such as a **chest x-ray** (6). A similar method is lung **ultrasonography** (6).

Microbiological and laboratory tests can be used, especially for bacterial pneumonia infections (6). **Gram stains**, **cultures**, and **molecular tests** (ex. PCR, urinary antigen tests, etc.) are options in this category (6).

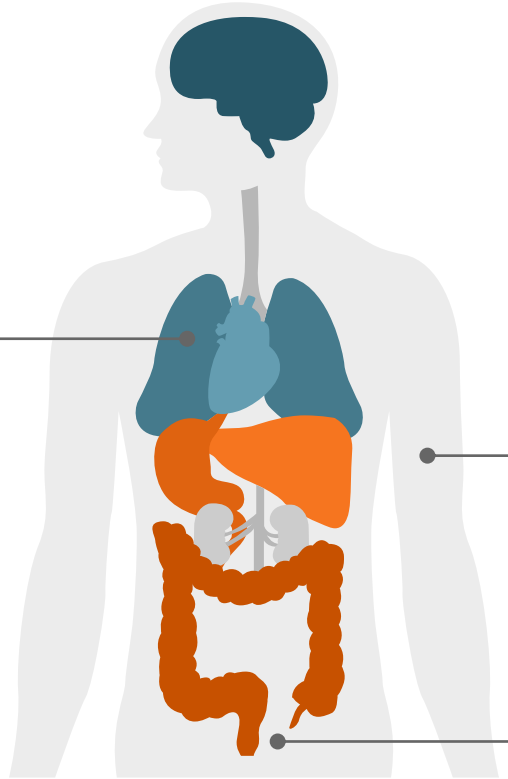
What samples are taken for pneumonia testing?

01

Respiratory Sites

These include:

- Lung aspirates (7)
- Lower respiratory tract samples: non-invasive, come from site of infection (7)
- Sputum samples (7)
- Upper respiratory tract samples (7)



02

Blood

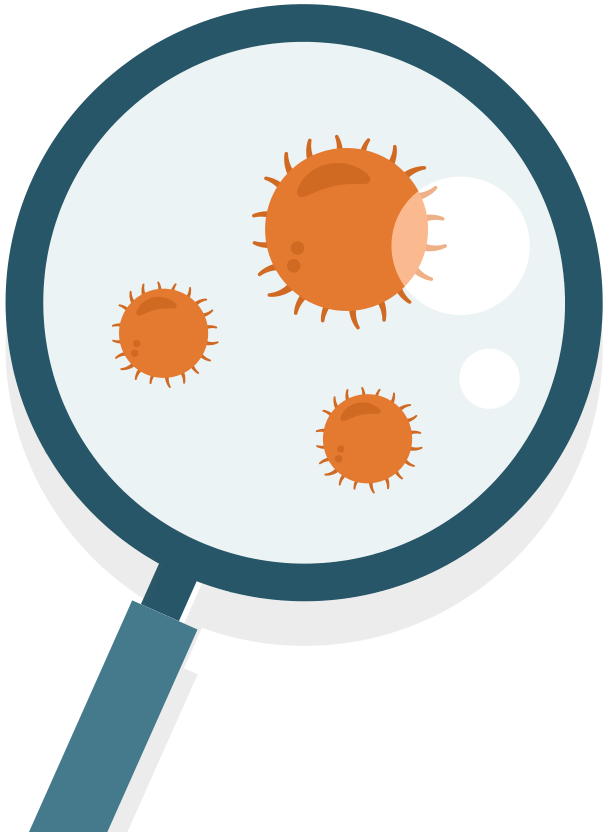
- Blood samples collected for culture in severe cases of community- or hospital-acquired pneumonia (8)

03

Urine

- Urine samples collected for suspected *S. pneumoniae* or *Legionella* infections (7)

Additional Testing for Pneumonia



- Additional testing can be performed in certain situations (9)
  - If patients are at risk for infection with unusual pathogens (10)
  - If patients are not responding to treatment (10)
  - If additional testing is likely to change antibiotic management (10)
- Tests include: **Ultrasonography/computed tomography (CT)** in certain situations; blood tests (9)
- **Routine HIV testing** should be offered in cases of community acquired pneumonia (CAP) (9)

Common Tests for *Legionella*

01	**Culture**	Grows colonies on buffered charcoal yeast extract (BCYE) agar; able to detect all species and serotypes of *Legionella* (11).
02	**Direct Fluorescent Antibody (DFA) Staining**	Antibodies specific to *Legionella* antigens are tagged with fluorescent dye (11).
03	**Urine Antigen Test (UAT)**	Shed antigen in urine is tested using an immunoassay; results are only reliable for serogroup 1 (11).
04	**Serological Testing**	Serum is tested for presence of antibodies; need to test both acute and convalescent phase serum samples (11).
05	**PCR**	Detects certain gene sequences; able to detect all species and serotypes of *Legionella* (11).

Culture

Test Characteristics:

- **Sensitivity:** ~99% (11)
- **Specificity:** variable – depends on sample used (11)
- **Speed:** results not available for at least 3-5 days, can be up to 2 weeks (11)
- Gold standard diagnostic test (11)
- **Requires special media** – standard is BCYE (11)

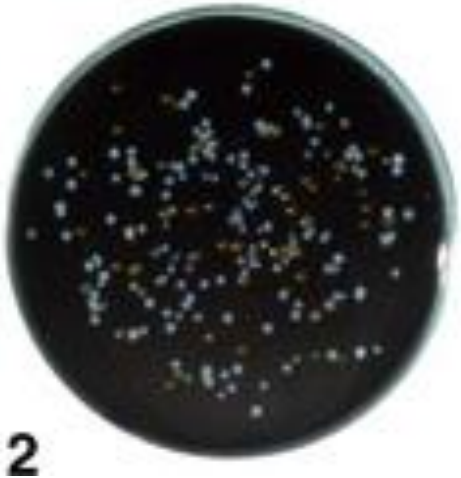


Figure 1. PCV agar with numerous *Legionella* colonies (12).

Expected results:

- Detection of colonies for **positive** diagnosis of *Legionella* infection (11)
- Appearance of *Legionella* colonies:
  - Convex and round with entire edges (12)
  - Center of colony typically bright white in colour (12)
  - May include long, filamentous forms, particularly after growth on agar (13)

Direct Fluorescent Antibody (DFA) Test

Test Characteristics:

- **Sensitivity:** ~70% (11)
- **Specificity:** 94 (11)-99% (14)
- **Speed:** results can be available within a few hours (11)
- False positives possible due to cross-reactions with other bacteria (11)
- Generally, positive DFA tests without other supporting evidence is not sufficient in *Legionella* diagnosis (11)
    - Therefore used as **confirmatory testing** for cultures (11)

Expected results:

- Antibody-sample binding resulting in fluorescence is **positive** for *Legionella* infection (15)

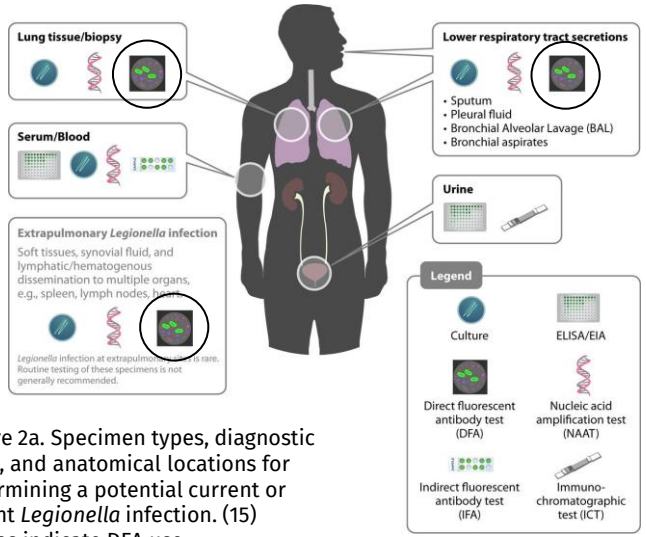


Figure 2a. Specimen types, diagnostic tests, and anatomical locations for determining a potential current or recent *Legionella* infection. (15) Circles indicate DFA use.

Urine Antigen Test (UAT)

Test Characteristics:

- **Sensitivity:** 69-100% (14)
- **Specificity:** >99% (14)
- **Speed:** fast, with results being available within minutes (14)
- Most common diagnostic test for *Legionella* infection (11, 14)
- Use monoclonal antibodies that specifically recognize most *L. pneumophila* **serogroup 1** antigens; do not recognize other serogroups or other *Legionella* species (14)

Expected results:

- Urine samples that produce absorbencies 3x greater than negative controls are **positive** for *Legionella* infection (16)

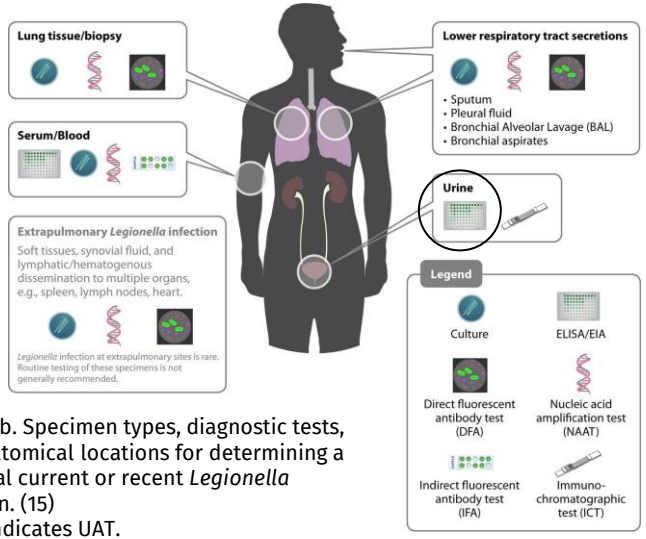


Figure 2b. Specimen types, diagnostic tests, and anatomical locations for determining a potential current or recent *Legionella* infection. (15)
Circle indicates UAT.

Serological Testing

Test Characteristics:

- **Sensitivity:** 17-100% (11)
- **Specificity:** >95% (11)
- **Speed:** seroconversion can take weeks, meaning tests are slow and not useful for clinical decision-making (11)
- Test for IgG, IgA, and IgM antibodies against *Legionella* (11)
- Major limitations
    - Seroconversion takes weeks, not useful for clinical decision-making (11)
    - Cannot accurately detect all species and serogroups (11)
    - Must use both acute and convalescent samples as single serum testing is not useful (11)

Expected results:

- In indirect immunofluorescence tests, 4x or greater increase in reciprocal antibody titer to ⩾128 is considered **positive** for *Legionella* infection (11)

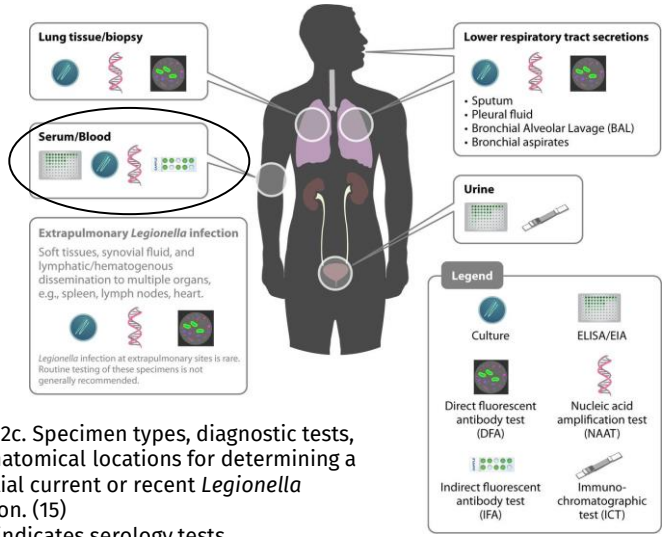


Figure 2c. Specimen types, diagnostic tests, and anatomical locations for determining a potential current or recent *Legionella* infection. (15)
Circle indicates serology tests.

PCR

Test Characteristics:

- **Sensitivity:** 17-100% (14)
- **Specificity:** 95-100% (14)
- **Speed:** fast, with results generally being available within hours (14)
- Amplifies specific ranges of *Legionella* DNA (14)
- Mostly used in research laboratories (14)
- Results rarely identify specific subgroups or serotypes (14)

Expected results:

- Amplification of the target DNA sequence (14)
    - Example target DNA sequences include a 386-bp portion of the *Legionella* 16S rRNA gene (17)

Figure 3. A PCR Machine schematic (18).

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