CASE 3 – THE MICROBIOLOGY LAB & *LEGIONNAIRES' DISEASE*

ADAPTED FROM WRITING & IMAGES AT:
HTTP://WIKI.UBC.CA/COURSE:PATH417201 7W2/CASE_3

Introduction

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1. Other than the stated bacterial cause, what are the **most common bacterial pathogens** associated with this type of infectious scenario.

2. What are all the **samples** that could be taken for laboratory testing (including the blood and ‘sputum’ in this case) and how important is the Microbiology Laboratory in the diagnosis of this particular infectious disease?

3. Explain the **tests that will be performed** on the samples in order to detect any of the potential bacterial pathogens causing this disease.

4. What are the **results** expected from these tests allowing for the identification of the bacteria named in this case.

The Case: A Cruise Holiday

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. Even more than the rest of his family, Tom enjoys the various hot tubs aboard the massive ship those first few day.

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. The cruise doctor examines Tom, notes his high temperature, relatively 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 a pneumonia. She explains that her presumptive diagnosis is that of Legionnaires disease and leaves Tom’s wife with a sterile sample container to collect whatever fluid Tom might cough up for delivery to her. She explains that she can do a microscopic examination on the respiratory fluid which will help in the diagnosis.

She starts Tom on erythromycin. More people are diagnosed with a similar pneumonia over the next two days, mostly in people who came aboard with a slightly compromised immune system. The cruise ship alerts the hospital at their next port of call in case any of the patients worsen enough to require hospitalization. When they arrive at port blood samples are collected from all of the patients and delivered to the hospital laboratory for serology. The ship also takes extra time in port to allow for a full scale sterilization regime to be performed on all of the hot tubs. At this stage Tom is feeling well enough to continue on the cruise.

1.Most common bacterial pathogens

Community-Acquired Pneumonia, referring to pneumonia in an individual who has had little contact with health care settings, can have a number of causes. Tom's increased hot tub exposure, characteristic symptoms and asthma are the biggest clues pointing towards Legionnaires' Disease.

Typical Pneumonia

Symptoms: rapid onset of fever and chills, presence of pleuristic chest pain, cough (productive).

Signs: elevated total leukocyte count, C reactive protein and erythrocyte sedimentation rate. Chest radiograph often shows lobar or segmental homogeneous opacity

Common Causes: *Streptococcus pneumoniae, Staphylococcus aureus, Haemophilus influenzae*, *Chlamydia pneumoniae*, *Pseudomonas aeruginosa*, and *Mycoplasma pneumoniae*

Atypical Pneumonia

Symptoms: body aches, fever (without chills), headache, cough (unproductive).

Signs: normal to slightly elevated total leukocyte count, C reactive protein and erythrocyte sedimentation rate. Chest radiograph often showing shadows (diffuse patchy or ground glass shadows)

Common Causes: *Mycoplasma pneumoniae, Legionella pneumonophila* (the presumptive diagnosis), and *Chlamydia spp.*

I.Most common bacterial pathogens

Streptococcus pneumoniae

-Gram + anaerobe

-Mode of entry: aerosol inhalation or bloodstream

-Risk: Bacteria may colonize a healthy person's respiratory tract asymptomatically but has a higher chance of becoming pathogenic in immunocompromised individuals

-Firmly adheres to alveolar epithelium and secretes pore forming toxic pneumolysin

Staphylococcus aureus

-Gram +

-Mode of entry: inhalation, open wounds, indwelling medical devices

-Risk: hospitalized patient as bacteria is a major cause of nosocomial infections, infection observed in healthy individuals too

-Produces alpha-hemolysin, a pore forming cytotoxin

Haemophilus influenza

-Gram - anaerobe

-Mode of entry: inhalation, transmission from contact with respiratory droplets

-Risk: infections established in individuals with weakened immune systems (e.g. children, elders or people with pre-existing conditions), can colonize upper respiratory tract of healthy persons without illness

-Capsule renders bacteria extremely resistant to phagocytosis

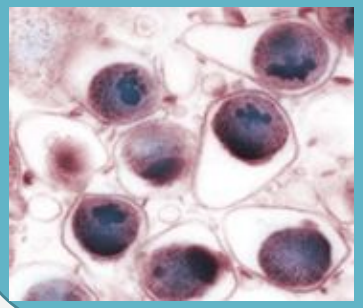
I.Most common bacterial pathogens

Chlamydia pneumoniae

-Gram - anaerobe

-Particularly long incubation period (3-4 weeks)

-Risk: Most hosts are susceptible, though infection is usually seen in school-age children



-Bacteria multiply without alarming the host

Pseudomonas aerugionsa

-Gram – opportunistic bacteria

-Mode of entry: most likely due to some epithelial damage or bloodstream deposition

-Risk: Very common cause of nosocomial infections, immunocompromise vulnerable

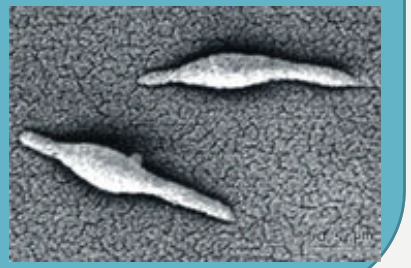
-Antibiotic resistance becoming and increasing worry

Mycoplasma pneumoniae

-Mode of entry: direct contact with respiratory fluids, extremely contagious and produces atypical pneumonia

-Risk: Most may be at risk due to quick spread in crowds

-Produces CARDS toxin among others and persists by avoidance of immune response

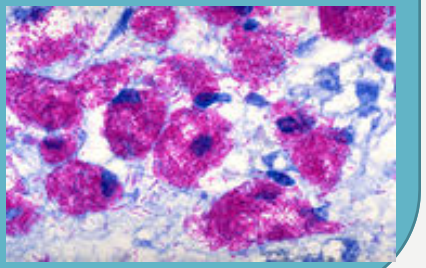


Mycobacterium avium complex (MAC)

-Comprised of *M. avium* and *M. intracellulare* or *M. Fortuitum*

-Risk: Immunocompromised hosts, particularly those with HIV are extremely vulnerable

-Can cause systemic fatal infections that can be treated with combination antibiotics over 12 month course



2. Types of Samples

Clinical presentation, patient history, physical exam and imaging are non-specific in a case of Legionnaires' Disease. The microbiology lab is crucial to determining the causative agent of the pneumonia. The following samples can be taken:

Lower Respiratory Tract (LRT) Secretions

Sputum is commonly collected to diagnose pneumonia

Timing – samples can be collected any time, preferably before treatment commences

Collection – patient will rinse their mouth, be asked to cough and expectorate sputum into a sterile container

Handling – if the specimen will be plated in a few hours, store at 2-5° C or -70° C for long storage

Blood samples are a routine method of diagnosing infectious diseases and help determine the most effective antibiotic treatment

Collection – small volume samples are taken following proper phlebotomy techniques

Handling – samples should be kept at 4° C or -80 C for long-term storage

Urine samples analyzed by antigen detection tests are especially useful for detecting *Legionella pneumophila*

Timing – samples should be collected within 7 days of onset of symptoms

Collection – 10-20mL should be collected directly into a sterile container

Handling – keep samples at 4° C and transport on wet ice or freeze at -20 - -70° C, which can remain stable for months

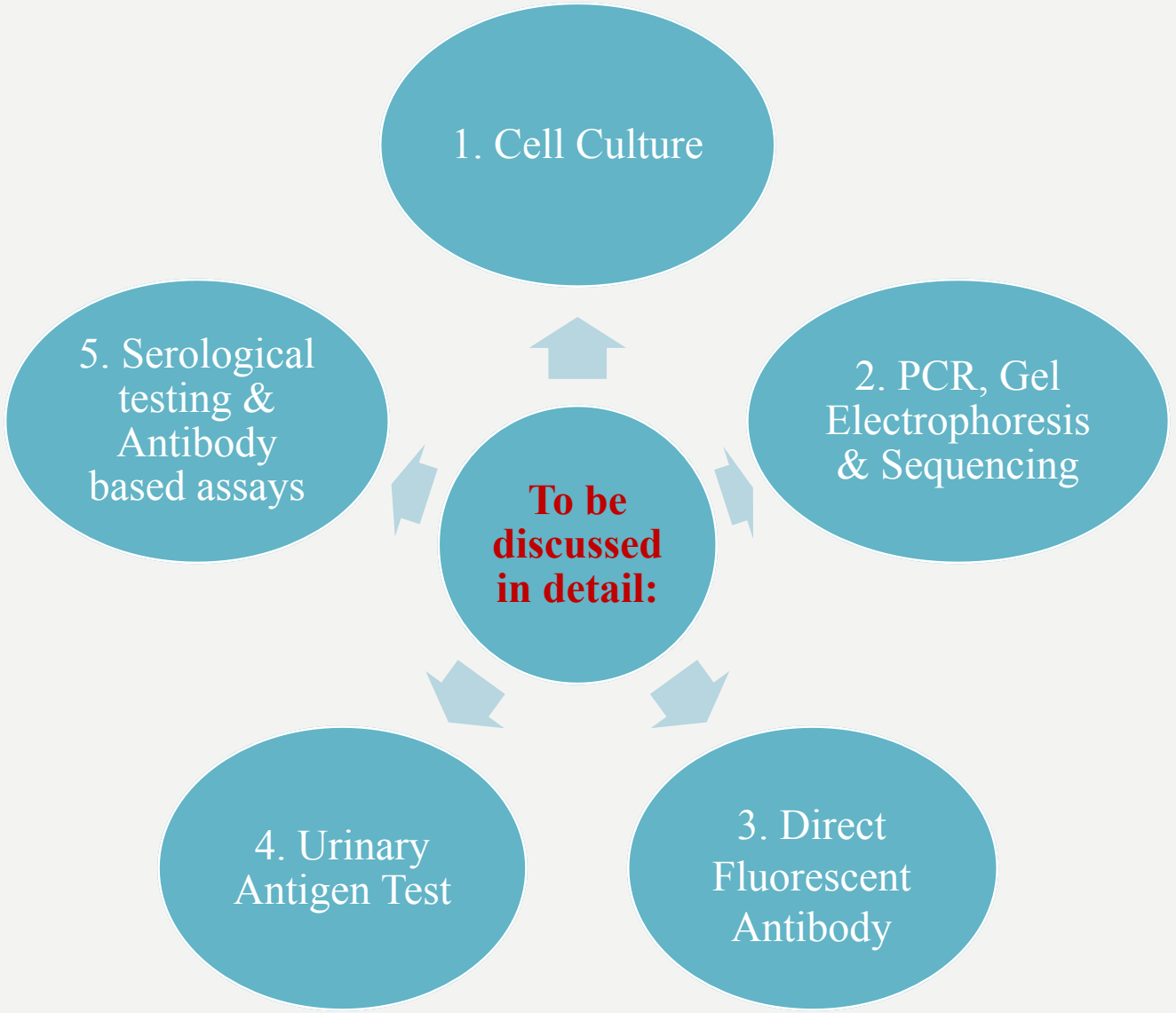
Other samples include (though may be impractical for different reasons)

Lung aspirates – Collected percutaneously in a multi-step procedure

Pleural aspirates – Useful in cases that are complicated by pleural effusion

Exhaled Breath Condensate (EBC) – No current standardized method for collection

3 & 4. Types of tests and the results



3 & 4. Types of tests and the results

Bacterial Cell Culture

1. **Initial Gram stain** is done to confirm presence of gram negative, small, filamentous rods (characteristic features of *Legionella*). MacConkey agar is used commonly to isolate gram negative bacteria.

*Note: Cell culture is routinely performed in these cases though has some limitation due to the time it takes to receive results

2. If confirmed to be Gram negative, the sample is incubated at 35° C on an agar plate with buffered charcoal yeast extract and α-ketoglutarate that provides iron and L-cysteine for *Legionella* growth. Selective pressures against normal flora (e.g. Cycloheximide, polymyxin B and vancomycin, which are toxic but do not inhibit L. pneumophila) may be used to increase chance of its recovery.

3. The bacteria will be observed in 3-5 days in colonies that are 05.-1mm in diameter, mostly white, smooth and convex. *L. micdadei* and *L. maceachernii* will appear blue, *L. pneumophila* will appear green, and *L. bozemanii* is white-gray to blue-gray and will fluoresce blue-white under long wave UV light.

Legionella spp. colonies under UV light

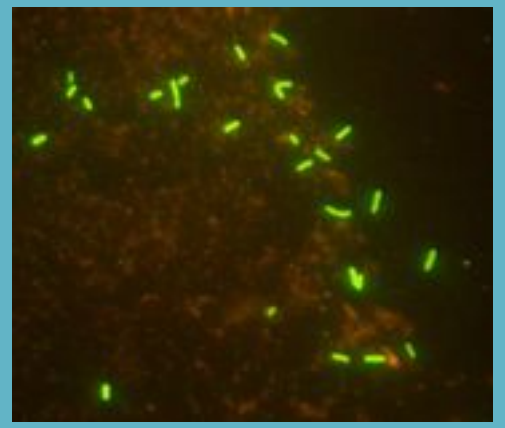
3 & 4. Types of tests and the results

Direct Fluorescence Antibody

Rapid microscopic procedure performed after cell culture, though it cannot be used alone to identify Legionella due to chance of false positives and negatives

1. Samples (may be from sputum, LRT secretions, tissue or blood) are placed on a plate in 10mm diameter circles and incubated with fluorescent tagged monoclonal antibodies specific for Legionella species and serogroups

2. In ~2-4 hours unbound antibodies are rinsed off before assessing the sample under microscope. Greater than 25 fluorescing rods is a positive result for *Legionella* infection.



Legionella DFA

3 & 4. Types of tests and the results

PCR, Gel Electrophoresis and Sequencing

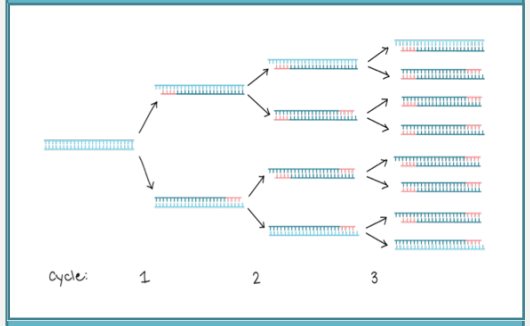
1. Primer of known *Legionella* genes (e.g. those for ribosomal RNA genes: 16S rRNA, 5S rRNA & 25S rRNA) and a DNA polymerase are added to the specimen (sputum, BLF, serum or urine).

Target	Sequence	Product size (bp)	Ref
16S rRNA	GCTAATCTTAAAGCGCC CCTGGCTCAGATTGAACG	212 bp	11,12,13
Mip	GCATTGGTGCCGATTTGG GCTTTGCCATCAAATCTTTCTGAA	168 bp	9,12

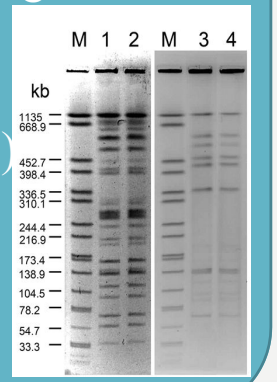
From: https://www.researchgate.net/figure/Primers-for-PCR-amplification-of-Legionella-spp_tbl1_259209534

2. Sample is heated to 94° C (to denature DNA) ⇨ temperature reduced to 40-60° C (for primers to anneal to DNA) ⇨ temperature raised to 70-74° C for DNA elongation.

The process is continued until the regions of DNA are at detectable levels.



3. PCR product is run through an agarose gel for 2-3 hours then visualized under UV light. Comparing the resulting bands to known sizes of DNA fragments can confirm Legionnaires' Disease. Threshold values can also be used (i.e. if threshold is reached before 50 cycles then genes of interest are in abundance)



4. The DNA is sequenced to confirm the presence of *Legionella* DNA (most likely by Sanger sequencing methods). With the NCBI BLAST, the results can be compared to sequences in the database; statistically significant matches allows inference of homology.

3 & 4. Types of tests and the results

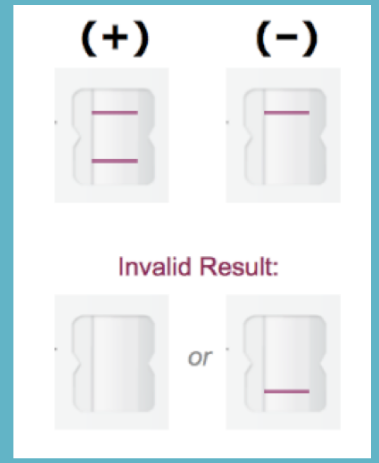
Urinary Antigen Test

A rapid and efficient method (sensitivities of 70-100% for *L. pneumophila* serogroup 1) that can be used even after antibiotic treatment has commenced. Available as an immunochromatographic test (ICT) in strip form or an ELISA.

1. In the test card, a membrane coated with rabbit antibody specific for *L.pneumophilla* serogro up 1 antigen and with controlled antibody is combined with rabbit anti-Legionella pneuphila serogroup 1 antigen + antispecies conjugates. A swab of the urine sample is placed on the sample pad. Positive control (w/ heat activated bacteria) and negative control swabs are provided.

2.After the swab, the sample flows toward the release pad and membrane and a result will be available in 15mins.

3.A positive result will 2 show pink lines in the control and sample zones (any visible sample line is positive).A negative result will show only one control line.



From: https://www.alere.com/en/home/product-details/binaxnow-legionella.html

3 & 4. Types of tests and the results

Serological Tests:

- Indirect fluorescent antibody test (detects IgG, IgM & IgA)
- Micro-ELISA
- ELISA (kits specific for *L. pneumophila* IgM & IgG)
- Counterimmunoelectrophoresis
- Microagglutination

→ Positive result: High fluorescent concentration

A schematic of how the discussed tests relate: