université PARIS-SACLAY

GRADUATE SCHOOL
Health and
Drug Sciences

M1 Development of Drugs and Health Products

OTU 01

Diagnosis of bacterial infections

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Aim and organization of the seminar

To understand the strategy of microbiological diagnosis of bacterial infection, and to discuss on several techniques, their advantages and drawbacks, their sensitivity and specificy regarding the pathology and the sample analyzed.

First part: examples of the microbiological diagnosis of two important clinical syndromes

Second part: group work on documents dealing with specific diagnostic techniques following by a short oral presentation (graded)

General principles

The first step of the diagnosis of an infectious disease is a clinical diagnosis, based on the presence of general and/or specific clinical signs

Clinical signs of infections		In which infection?
General	Fever (moderate or high)	Most of them
	Tiredness	Most of them
Specific (examples)	Cough	Bronchopulmonary infections
	Diarrhea	Gastroenteritis
	Vomiting	Gastroenteritis Meningitis (violent vomiting)

In some cases, an etiological diagnosis (= identification of the pathogenic microorganism) is needed:

Etiological diagnosis

- **Why?**
  - To identify precisely the responsible micro-organisms in order to adapt the antimicrobial treatment
  - For epidemiological suveillance
- **When?**
  - In severe infections or in moderately severe infections due to frequently antibiotic-resistant bacteria
  - In recurrent infections
  - In rare infections
  - When a differential diagnosis is needed
- **How?**

The first step of an etiological diagnosis?
Sampling of the appropriate specimen to be analyzed

Sample processing and the techniques used on it vary according to several criteria, the first of which is whether the sample is mono- or polymicrobial ?

Which samples are monomicrobial, which are polymicrobial?

	Monomicrobial	Polymicrobial
Blood	X	
Cerebrospinal fluid	X	
Skin		X
Sputum		X
Stool		X
Joint fluid	X	
Urine	X	

Which strategies can be used for microbiological identification?

Direct diagnosis

=

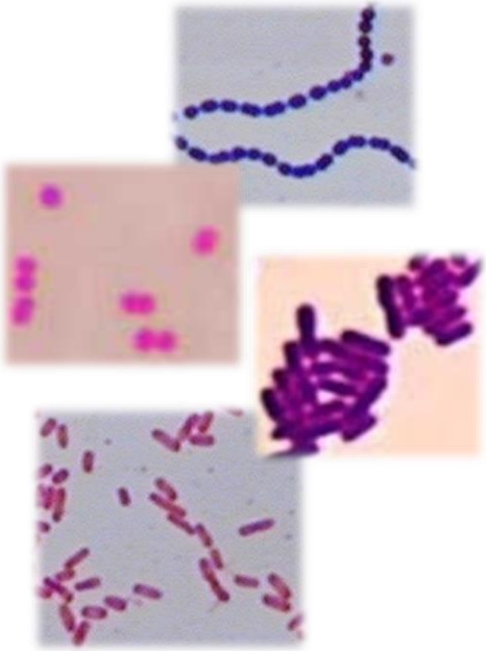
identification of the pathogenic microorganism

Indirect diagnosis

=

identification of the host immune response raised against the pathogen

Rarely used in bacterial infections



Direct diagnosis

Microscopic examination of samples
(after appropriate staining)

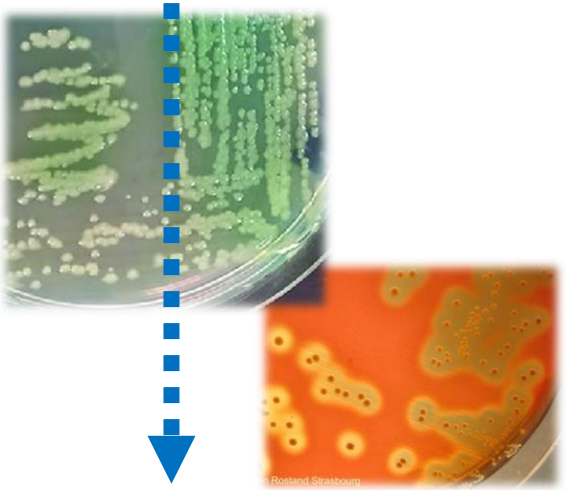
Bacterial culture appropriate growth media

\+

Identification of the viable bacteria
(biochemical testing, MALDI-TOF analysis)

\-

Identification of a specific component of the bacteria
(genomic, antigenic)

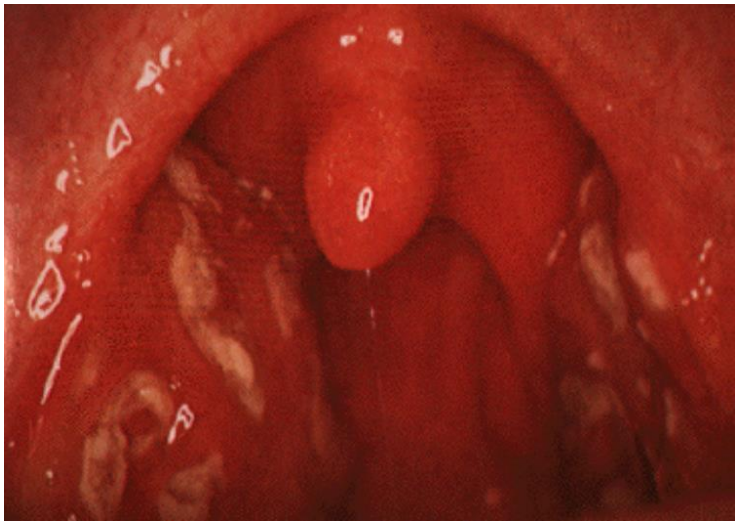


Diagnosis of important clinical syndromes

1- GAS PHARYNGITIS

Pharyngitis is characterized by inflammation of the pharynx and/or tonsillar tissues

- Mainly caused by viral agents (≈80% in adults, ≈60% in children)
- Main causative bacterial agent: *Streptococcus pyogenes* (GAS)



The main clinical symptoms are:

- Sore throat
- Odynophagia
- Fever

common features independent of the etiology

Clinical differentiation between etiologies quite difficult

Sykes et al., Can Fam Phys, 2020

Estimated number of cases of GAS pharyngitis in children: 450 million/year worldwide

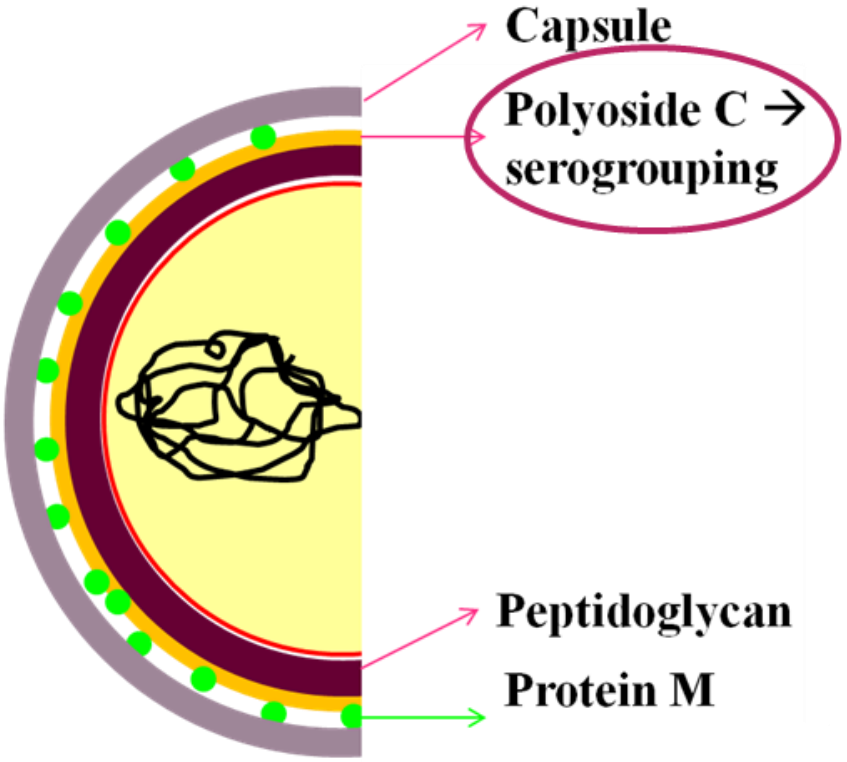
Most of pharyngitis are self-limiting without sequelae, but GAS infections can have rare but life-threatening complications:

- Nonsuppurative complications (mainly acute rheumatic fever, potentially leading to chronic heart failure)
- Suppurative complications (such as face cellulitis)

Preventing complications requires antimicrobial treatment,
but growing antibiotic resistance has emphasized the urgent need to minimize antibiotic use.

To limit antibiotic use to GAS pharyngitis, a biological diagnosis is needed

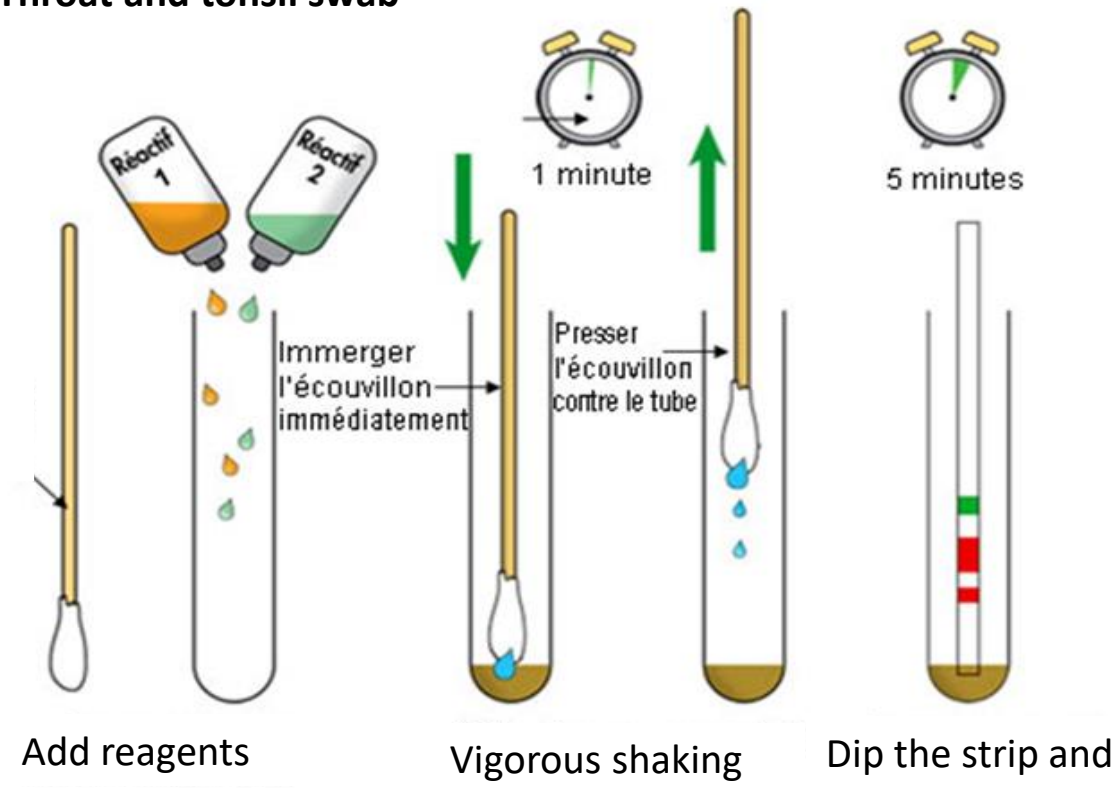
Rapid antigen detection testing (RADT)



Schematic representation of the SGA surface

Detection of the Lancefield type A polyoside with an enzyme immunoassay test (RADT)

Throat and tonsil swab



NEGATIVE : One red line appears in the control region (C). No apparent red or pink line appears in the test region (T).

POSITIVE : Two distinct red lines appear. One line should be in the control region (C) and another line should be in the test region

Sensitivity ≈ 85%
Specificity ≈ 96%

Cohen et al., Cochrane Librairy, 2016

For which patient should this RADT be used? In France:

- In infants between 3 and 15 years old (GAS infection is very uncommon in children younger than 3y)
- In adults with a McIsaac (clinical) score ≥ 2

Predictive algorithms allow to guide investigation and prevent antibiotic overprescribing by assigning signs and symptoms an aggregated pretest probability for bacterial pharyngitis.
The McIsaac score is the most widely used method.

MODIFIED CENTOR CRITERIA: "STREP"	POINTS
Sans cough (absence)	1
Tender, swollen anterior cervical lymph nodes	1
Right age	
• 5-14 y*	1
• 15-44 y	0
• ≥ 45 y	-1
Exudates (tonsillar)	1
Pyrexia (temperature > 38°C)	1

European guidelines recommend relying on negative RADT results without culture confirmation

But guidelines may be slightly different in other countries…

In some situations, throat cultures are required:

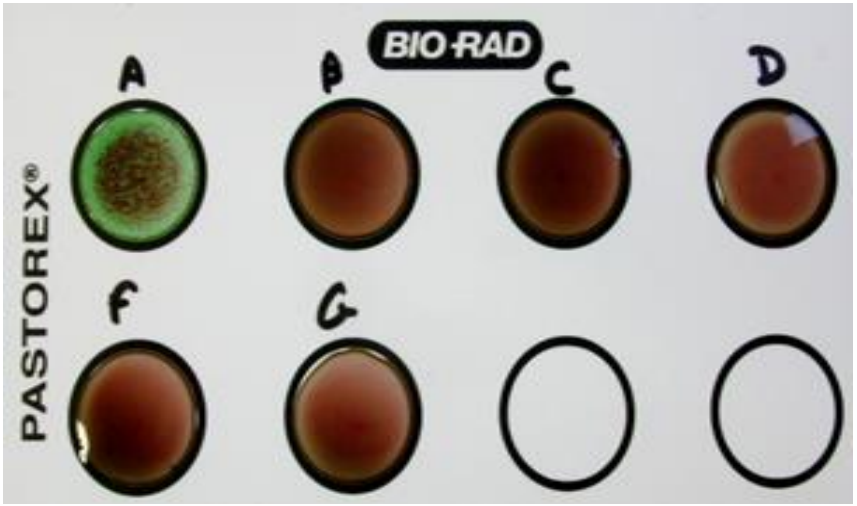
- Patients allergic to beta-lactams antibiotics
- Patients with recurrent pharyngitis

Cultures on blood agar plates

Detection of beta-haemolytic colonies

Identification of colonies

- By serogrouping (type A polyoside)
- By MALDI-TOF mass spectrometry

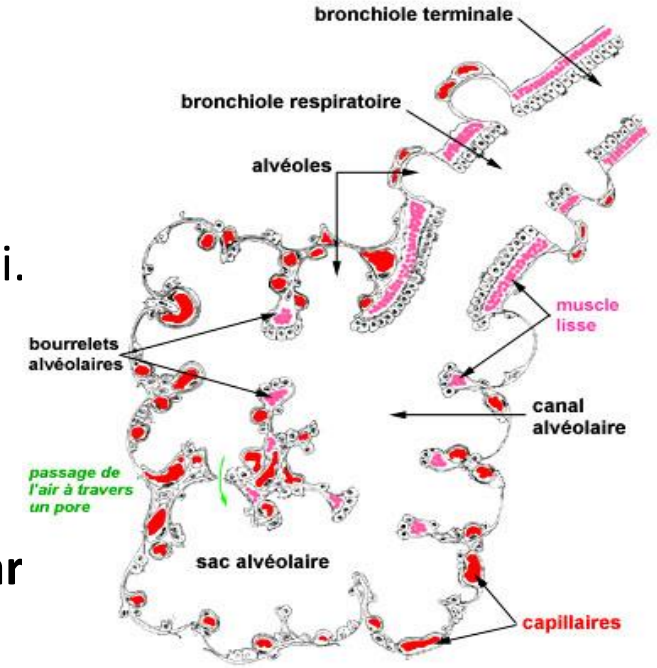


Diagnosis of important clinical syndromes

2- COMMUNITY-ACQUIRED PNEUMONIA (CAP)

Pneumonia is an acute infection of the lung parenchyma that causes them to function abnormally. It is caused by a wide variety of microorganisms: bacteria, viruses, and fungi.

Pneumonia can be classified as typical (due to pyogenic microrganisms multiplying in the pulmonary alveoli) **or atypical** (due to intracellular microorganisms infecting the pneumocytes) **but the clinical presentations may be similar**



The main clinical signs are :

- Cough
- Fever, chills, tachycardia, dyspnea (tachypnea)
- Tiredness

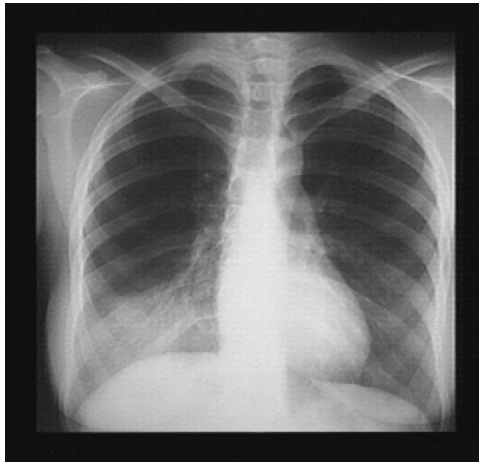
Pneumonia is consistently among the leading causes of morbidity and mortality worldwide

S. pneumoniae is the most commonly isolated bacterial pathogen, accounting for more than 25% of cases of CAP worldwide.

Main bacterial aetiologies of CAP

Typical pneumonia | **Atypical pneumonia**

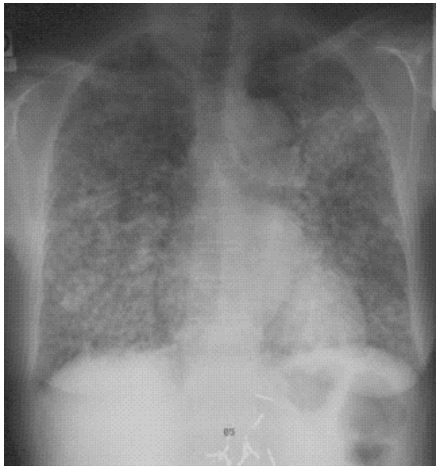
Chest radiography imaging



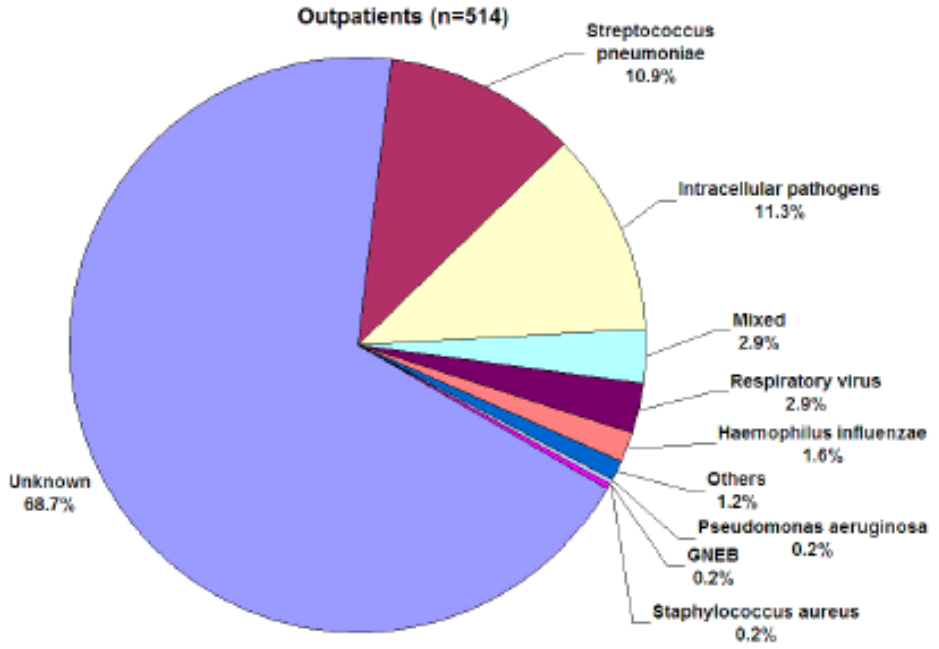
Typical pneumonia (pneumococcus)

-**To assert the diagnosis of CAP** and to direct towards an typical or atypical one.

- **To detect elements of severity** of the infection and/or **complications.**



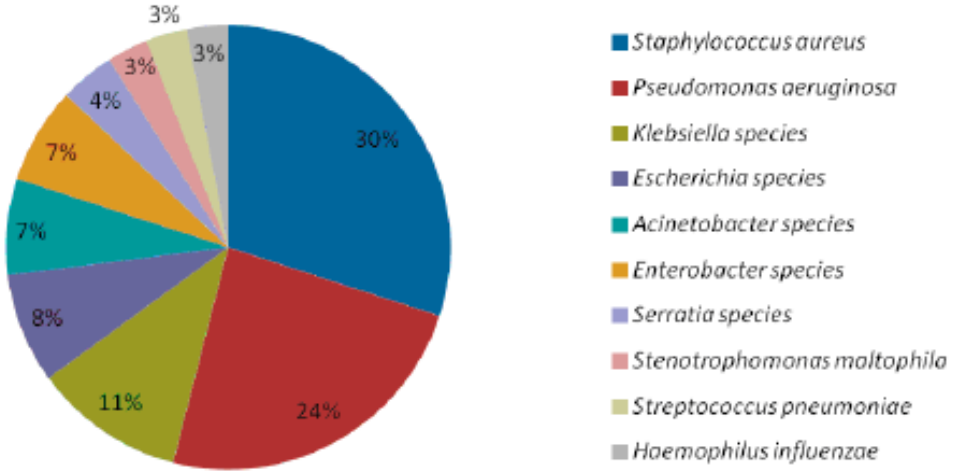
Atypical pneumonia



(A): abbreviations: GNEB = Gram-negative enteric bacilli.

The overall rate of pathogen detection among patients with CAP is 30% to 40% (after classical bacterial culture on synthetic medium)

Most commonly identified pathogens among adult outpatients with CAP, in Spain. The pneumococcus is the major etiology, as in patients admitted to wards or to intensive care unit *(Cilloniz et al. Thorax, 2011)*



Most commonly identified pathogens in patients with hospital-acquired pneumonia.
(SENTRY study)

Routine microbiologic testing of outpatients with CAP is unnecessary. Testing may be indicated according in some particular cases (frailty patients or specific clinical signs suggesting a high severity of the CAP)

Sample and diagnosic testing in pneumonia (after chest radiography for all patients)

Cilloniz et al. Int J Mol Sci, 2016

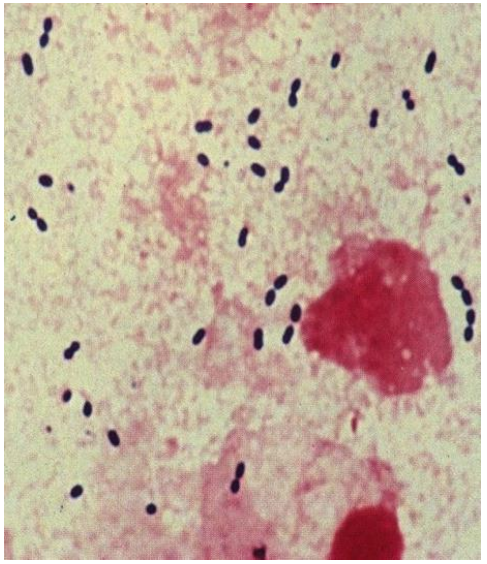
Collected before probabilistic antibiotic treatment

Condition of Pneumonia	Blood Cultures	Respiratory Samples	Urinary Antigen Test for Legionella/ Pneumococcus
Outpatient	**Most outpatients with community-acquired pneumonia do not require microbiologic testing and can be treated empirically**		
Hospitalized patients (ward)	×	**Sputum**	×
Hospitalized patients admitted to ICU	×	BAL/BAS in intubated patients	×
Failure of outpatient antibiotic treatment		Sputum culture	×

BAL: bronchoalveolar lavage; BAS, bronchoaspiration

Sputum analysis

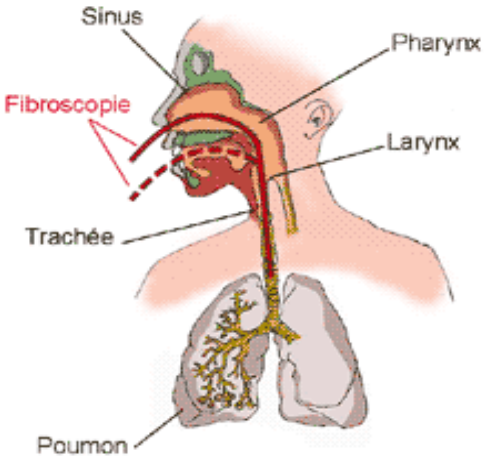
- A good quality sample is considered when the sputum sample **contains less than 10 epithelial cells** (minor saliva contamination) **and more than 25 lymphocyte cells** (lung inflammation).
- **Gram staining**
- **Liquified sputum should be diluted before plating on appropriate agar plates** (blood, Polyvitex, MacConkey or Drigalsky)
- **Identification of bacteria detected above the threshold of 10^7 CFU/ml** (metabolic, antigenic, MALDI-TOF identification)



Protected respiratory samples

Example: BronchoAlveolar Lavage

- BAL threshold: 10^4 UFC/ml



❑ Respiratory samples

- **Respiratory sample collection should be performed before patients initiate antimicrobial therapy.**
- **To distinguish colonization from infection, quantitative threshold have been defined, depending on the sample collected**

❑ Other tests?

Blood cultures, since pneumococcal pneumonia are bacteraemic in 20% of cases

Urinary antigen tests

Molecular techniques

WORK IN GROUP

Constitution of 3 groups (4, 4 and 5 students)

Per group:

- collective analysis of scientific articles on a diagnostic technique
- preparation of a short summary for an oral presentation, with predefined questions to be answered

Please, put a label on the table with your name