Non-cultural bacterial diagnostic methods

- **Antigen detection.** e.g. latex agglutination

- **Antibody detection.** e.g. agglutination tests, complement fixation tests, direct or indirect immunofluorescence

- **Molecular methods.** such as Polymerase Chain Reaction (PCR)
SEROLOGICAL METHODS:

There are two methods:

1. Identification of an organism (unknown antigen) with known antiserum:

   - **Capsular swelling (Quelling) reaction:** The capsule swells up when comes in touch with specific antiserum. Reaction is positive with *Streptococcus pneumoniae*, *Homophiles influenza*, *Niesseria meningitides*.

   - **Slide agglutination test:** Used to identify Salmonella & Shigella, looking for O, H, & Vi antigens.

   - **Latex agglutination test:** The test is used in diagnosis of *H. influenzae*, *N. meningitidis*, *Cryptococcus neoformans* (yeast).

   - **Counter immunoelectrophoresis test:** The unknown bacterial antigen and the known specific antibody move towards each other and form a precipitate. The test is used to diagnose CSF pathogens, e.g.: *H. influenzae*, *N. meningitidis*, *S. pneumoniae*.

   - **ELISA:** Used to diagnosis *Cotynebacterium diphtheria* infections (Diphtheria)- Meningitis

   - **Fluorescent - antibody test:** the known antibody is labeled with a fluorescent dye & detected by an U.V.microscope, either directly or indirectly when antibody unites with antigen.
2. Identification of serum antibodies (unknown) with known antigens:

- **Slide & tube agglutination test:** Serial dilution is made for patient serum and then bacterial antigen is added. Highest dilution of serum with agglutination shows the titre.
  This test is to diagnose: enteric fever, brucellosis, plague and rickettsial diseases.

- **Cold agglutinin test:** Patients infected with *Mycoplasma pneumoniae* will develop autoimmune antibodies that agglutinate human RBC at 4°C but not at 37°C.

- **Seroological tests for syphilis:** Include:
  1. Non-treponemal tests: using cardiolipin antigen: Rapid plasma regain (RPR) and VDRL tests.
  2. Treponemal tests: such as immobilizing test
Classical bacterial identification can be performed on pure cultures of bacteria

**Isolation of Individual Bacteria**

Specimen is “streaked”, using a sterile loop, onto solid media. The agar plates (media) are incubated at appropriate temperature and atmosphere

- Often at 35°C.
- Often at 5% CO₂
- Usually first examined after 24 hours

“Streaking a Plate”

Isolation techniques include:

- Streak plate technique
- Pour plate technique
- Spread plate technique
Bacterial Isolation

(a) Mixed sample

(b) General-purpose nonselective medium (All species grow.)

(c) Selective medium (One species grows.)

(d) Loop containing sample

(e) Steps in a Streak Plate; this one is a four-part or quadrant streak.

(f) Steps in Loop Dilution; also called a pour plate or serial dilution.

Note: This method only works if the spreading tool (usually an inoculating loop) is sanitized (flamed) after each of steps 1-4.

(g) Steps in a Spread Plate

(h) 'Hockey stick'

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Growth of Colonies

- **Bacterial Colony**
  - Result of one bacterium being isolated from others during “streaking procedure”
  - That bacterium grows in numbers exponentially (colonies)
  - Some bacteria have rapid generation time of 20 minutes such as E. coli. Mycobacterium tuberculosis grow slowly.

**Colonies “Picking”** Sterile loop is touched to surface of colony and transferred to fresh, sterile media and incubation for 24 hours

**Now we have a pure culture of bacteria**
Testing is now done to confirm the identification of the bacteria culture

- **Stains**
- **Biochemical tests**
- **Serological tests (using known antibodies)**
- **Molecular tests (nucleic acid probes)**
Examples of Biochemical Tests

*Klebsiella pneumoniae* & *Staphylococcus aureus*
Gram-negative rods and gram-positive cocci

*E. coli* gram stain, gram negative rods

Antimicrobial Sensitivity Test

API20E Strip
Factors limiting usefulness of bacteriological investigations

- wrong sample (e.g. saliva instead of sputum)
- delay in transport sample/ inappropriate storage (e.g. CSF)
- overgrowth by contaminants (e.g. blood cultures)
- insufficient sample / sampling error (e.g. in Mycobacterial disease)
- patient has received antibiotics
Sensitivity tests

on solid media (disc diffusion technique)

in liquid media (minimum inhibitory concentration (MIC)- (MBC) tests

Breakpoint methods (E-test)

E-test