MIC-determination

- Definitions: MIC- and diffusion methods
- Standardisation
- Clinical breakpoints and epidemiological cut-off values
- MIC contra disk diffusion
- Sensititre
**Definitions**

**MIC methods**

MIC: Minimal Inhibitory Concentration
The lowest concentration that is able to inhibit growth of the bacteria

Two fold dilutions of antimicrobial → inoculate + incubate →
the lowest conc. with no visible growth is read as the MIC

- microdilution MIC (microtitre wells, Sensititre)
- agar dilution (also called plate dilution)
- macrodilution MIC (in tubes)

**Diffusion methods**

Agar plate is inoculated and antimicrobial diffuses from a disk or from a paperstrip in to the agar. Next day the growth inhibition zone is read.

- disk diffusion
- E-test
Standardisation

All methods are extremely sensitive to variations in performance. Many factors influence the result:

- Size of inoculum
- Contents and acidity (pH) of the broth or agar
- Incubation time and temperature
- Reading procedures

And further more for the diffusion methods:
- Growth rate of the bacteria
- Diffusion rate of the antimicrobial into the agar
- Depth of the agar
- Age of the agar

Reliable and reproducible data = Standardized methods and daily QC!
The CLSI standards

- International standard that describes the methods in details - media, inoculum, incubation, etc. - both disk diffusion and MIC - for example acidity 7.2-7.4 and agar depth 4 mm...

- CLSI has defined QC reference strains and the acceptable QC results for these strains.

- CLSI recommends clinical breakpoints for interpretation of the result

  **Resistant:** Treatment failure can be expected.

  **Sensitive:** Successfully treatment can be expected.

  **Intermediary:** Treatment is possible if the infection is in bodysites where the antimicrobial is concentrated. Primarily a bufferzone to avoid misinterpretation
Clinical breakpoints

• Are established according to
  - the serum conc. obtained with normal dosage
  - and the MIC distribution for the bacteria

• CLSI has not applied breakpoints for all antimicrobials and for all bacteria
Epidemiological cut off values

European counterpart to CLSI: www.EUCAST.org

- Uses CLSI methods, QC reference strains etc
- Defines species-specific clinical breakpoints
- Defines epidemiological cut off values
  - for monitoring purpose only, no relation to clinical data
  - defined by the WildType distribution of the bacteria
  - all isolates with MICs above the WT distribution are called resistant!
Illustration of epid. cut off values

No. of isolates

Epid. cut off value

Clinical breakpoint

WT

MIC-value
**Diffusion tests are fine...**

- If standardized methods are used and QC performed
- The methods are correlated to MIC by regression lines for each antimicrobial for both slow growing and fast growing bacteria.
# MIC contra agar diffusion

## MIC determination
- Golden standard for AST
- Data more reproducible
- Better separation of R/S
- More information
- Expensive
- Only pure cultures
- Contaminations more difficult to detect

## Diffusion methods
- Cheaper
- Primary material
- See contaminations
- Quick screening (4 hours)
- Qualitative information: “Resistant or not”
- Less reproducible data
- Standardisation more difficult
Sensititre-system

- Kommercially available microdilution MIC in microtitrewells, Trek Diagnostics, UK
- Two fold dilution of dehydrated antimicrobials – just add the bacteria susp.
- The Sensititre-system is standardized according to the CLSI standard and the system is validated by the FDA in the USA
- The system consists of:
  - MIC panels (custom designed or pre-designed by Trek)
  - nephelometer and MacFarland 0.5 standard
  - autoinoculator
  - tubes, dosing heads and broth
  - Sensitouch and software for reading the panels
  - Service visit by Trek Diagnostics once a year