

# Check list to facilitate implementation of antimicrobial susceptibility testing with EUCAST breakpoints

**Before implementing EUCAST breakpoints and antimicrobial susceptibility testing (AST) methods in the laboratory, consider the following:**

1. Liaise with the National Antibiotic (or Antimicrobial Susceptibility Testing) Committee (NAC).
2. Identify all AST methods used in the laboratory (disk diffusion, automatic device, gradient tests and others). Ensure that all methods are ready for implementation with EUCAST breakpoints.
3. Identify support systems that may be affected (laboratory accreditation, manuals, laboratory information system and mandatory reporting systems).
4. Identify a “champion” among laboratory staff. The champion will take responsibility for and be the lead person during the whole implementation process.
5. Liaise with a laboratory which has already implemented EUCAST susceptibility testing breakpoints and methods. Arrange for staff to visit.
6. Identify and inform all stakeholders (laboratory staff, customers/users, antimicrobial resistance surveillance programmes and distributors of materials and devices for antimicrobial susceptibility testing).
7. Make sure that necessary “AST materials” will be available. Check EUCAST web page table on [preparedness of manufacturers of AST materials](#).
8. Set up a 3–6 month educational programme within the laboratory with a pre-determined date for implementation.
9. Inform external quality assessment programme organisers.
10. Consult when necessary with EUCAST (contact information available at [www.eucast.org](http://www.eucast.org)).

## Issues with implementation of automated AST systems

1. Consult with the National Antibiotic Committee regarding any national issues.
2. Ensure that the system can provide support for EUCAST breakpoints for all required antibiotics. Ask the manufacturer to list discrepancies between the System and EUCAST recommendations – these may be different at different points in time and between countries and/or installations.
3. Note that an “IE” and a “dash” in the EUCAST tables are in lieu of breakpoints – a laboratory wishing to adhere to EUCAST recommendations should not substitute these with other breakpoints.

## Issues with implementation of the EUCAST disk diffusion method

The EUCAST disk diffusion method is based on a confluent inoculum (McFarland 0.5) on Mueller-Hinton agar with or without 5% horse blood and 20 mg/L  $\beta$ -NAD. It is important to follow the method description (available on [www.eucast.org](http://www.eucast.org)).

### Note the following significant details:

- **The inoculum suspension should be equivalent to a 0.5-McFarland standard**, preferably measured with a photometric device.  
Exception: *Streptococcus pneumoniae* is suspended to McFarland 0.5 from a blood agar plate, but to McFarland 1.0 from a chocolate agar plate.
- **The growth should be confluent and evenly spread over the plate.**  
A correct inoculum and satisfactorily streaked plates will result in a confluent lawn of growth and uniformly circular inhibition zones. The inoculum should be evenly spread over the agar surface to get reproducible zone diameters. To avoid too heavy an inoculum of Gram-negative organisms, take particular care to remove excess fluid from the swab by turning it gently against the inside of the tube before inoculating the plate.
- **Adhere to the 15-15-15 minute rule** to get reproducible results:
  - Use the inoculum within 15 minutes of preparation.
  - Apply disks within 15 minutes of inoculation of plates.
  - Start incubation within 15 minutes of application of disks.Small changes in current laboratory routines, such as setting up tests in smaller batches, may be necessary to adhere to the "15-15-15" rule.
- **Use the correct disk contents.** These are presented in EUCAST Clinical Breakpoint and Quality Control tables. Disk contents may differ from what has been previously used in the laboratory.

- **Do not shorten or prolong the incubation time beyond 16-20 h.**
- **Follow instructions for reading.**  
Zone edges should be read at the point of complete inhibition as judged by the naked eye. Read Mueller-Hinton plates without supplements from the back against a black background illuminated with reflected light. Read Mueller-Hinton plates with supplements from the front with the lid removed illuminated with reflected light.

### **Suggestions for implementation of the EUCAST disk diffusion method in the laboratory: A practical guide for the champion**

1. Educate laboratory staff in the EUCAST disk diffusion methodology with focus on inoculation of plates and reading zones. A slide show is available at [www.eucast.org](http://www.eucast.org).
2. Initiate the practical training by having all laboratory staff read zones from the same plate. The aim of the exercise is to harmonise the reading of inhibition zones in the laboratory. New staff should perform these exercises before entering routine bacteriological work.  
A reading guide is available at [www.eucast.org](http://www.eucast.org).
  - Start by reading zones for two control strains on Mueller-Hinton agar without supplements, e.g. *E. coli* ATCC 25922 and *S. aureus* ATCC 29213. Choose four antibiotics routinely tested with respective species. Repeat three days in a row. Compare the results between staff and between days and check that mean zone diameter values are close to the targets and that all values are within the quality control ranges. Quality control tables are available at [www.eucast.org](http://www.eucast.org).
  - Discuss the results during staff meetings. Repeat the exercise using the same control strains and antibiotics until everyone gets the same result.
  - Repeat the exercise with *P. aeruginosa* ATCC 27853 and *E. faecalis* ATCC 29212.
  - Repeat the exercise using MH-F (Mueller-Hinton agar with 5% horse blood and 20 mg/L  $\beta$ -NAD) with *H. influenzae* NCTC 8468 and *S. pneumoniae* ATCC 49619.
  - Repeat the exercise with a few clinical isolates of additional organisms, e.g. streptococci groups A, B, C and G. Compare individual results with the mean of the group.
3. The next step is the preparation of the inoculum and the seeding of plates. The aim is to prepare a standardised even and confluent, non-jagged, lawn of growth. Best results are obtained when using a simple

nephelometer/spectrophotometer to control the density of the inoculum. Whatever technique is used for inoculating plates (cotton swab with a plate rotator or manual swabbing in three directions) make sure that the resulting lawn of growth is even and edges are not jagged. Use a few antibiotic disks with sharp zone edges so that reading is not a problem.

Staff should repeat the process for all control strains recommended by EUCAST. Compare the results between staff and between days and check that mean zone diameter values are close to the targets and that all values are within the quality control ranges. Quality control tables are available at [www.eucast.org](http://www.eucast.org).

4. Before introducing the EUCAST disk diffusion into the routine work of the laboratory, run Quality Control strains daily (using all routinely used antibiotic disks) until the testing is compliant with EUCAST specifications and harmonised within the laboratory.

**Do you have questions about EUCAST disk diffusion method?  
Or do you need support implementing the disk diffusion test?**

Please contact [erika.matuschek@ltkronoberg.se](mailto:erika.matuschek@ltkronoberg.se)

**Or the EUCAST Secretariat**