Inoculum

When using the technique of Kirby-Bauer, the inoculum is standardized according to the method described by the CLSI (chapter 2 and 3), which results in confluent growth for bacteria and semi-confluent growth for most of the *Candida* species isolates.

Inoculum standardisation

Direct colony suspension method:
Suspend several morphologically similar colonies from an 18-24 h agar plate (nonselective) into 4-5 ml sterile saline solution, and then immediately adjusting the turbidity to match that of the $BaSO_4$ standard (0.5 McFarland). For *Candida* spp. that are subcultured onto blood agar or Sabouraud dextrose agar, five distinct colonies from a 24-hour-old culture are suspended in 5 ml sterile saline (0.145 mol/L; 8.5 g/L NaCl; 0.85% saline).

a) Within 15 minutes, dip a sterile cotton swab into the adjusted suspension and remove inoculum from the swab by exerting firm pressure on the inside of the tube.
b) Within 15 minutes swabs are used to inoculate the test plates.
c) Inoculate the dried surface of the appropriate agar plate by streaking the swab over the entire surface. Allow the surface to dry 3-5 minutes or maximum 15 minutes before applying Neo-Sensitabs to the media.
d) Select appropriate tablets, e.g., such as recommended by CLSI or EUCAST. Use no more than nine Neo-Sensitabs per 150 mm plate or four Neo-Sensitabs per 100 mm plate when testing *Candida* spp., *H. influenzae, N. gonorrhoeae,* and *Streptococcus* spp.
e) Neo-Sensitabs are dispensed onto the surface of the inoculated agar plate.

Incubation and reading of plates

a) Within 15 minutes, place the agar side up in a 35 °C (± 2 °C) incubator. For some strains special recommendations are noted in the tables e.g. *Haemophilus* spp., *N. gonorrhoeae*, *S. pneumoniae* and other streptococci should be incubated in an atmosphere enriched with 5 % CO_2.
Incubate no more than 5 plates in a stack. Plates in the middle of the stack will take longer to reach the desired incubation temperature than plates at the top and the bottom.

b) Examine the plates after 16-18 hours' incubation (20-24 h for *Candida* spp. *N. gonorrhoeae*, *S. pneumoniae* and other streptococci). Full 24-hour incubation is recommended for the detection of Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Enterococcus* spp. for vancomycin resistance. Hold the plate up to transmitted light and examine the oxacillin, linezolid and vancomycin zones for light growth (minute colonies) of methicillin, linezolid or vancomycin resistant colonies, respectively, within apparent zones of inhibition. Any discernible growth within the zone of inhibition is indicative of methicillin, linezolid or vancomycin-resistance. The edges of the zones of inhibition contain a large number of small colonies when using Trimethoprim, Sulphonamides and Trimethoprim + Sulfa-methoxazole tablets. In this case zones of inhibition are measured up to colonies of normal size (disregard slight growth and measure the more obvious margin).

c) For some antimicrobial agents (chloramphenicol, clindamycin, erythromycin and tetracycline) the zones of inhibition will contain a gradient of growth. In this case zone diameters should be read half-way between the start of inhibition and complete inhibition.

d) With *Proteus* spp. ignore the thin veil of swarming growth in an otherwise obvious zone of inhibition. The diameters of the zones of complete inhibition are measured as determined by gross visual inspection. Zones are measured to the nearest whole millimeter.

e) The measured zone diameters of inhibition are compared with the zone interpretative tables for the individual antibiotics in order to determine the agent(s) most suitable for use in antimicrobial therapy.