DTU Food
National Food Institute

Community Reference Laboratory Antimicrobial Resistance

PROTOCOL FOR MIC-TESTING WITH SENSITITRE
of *Staphylococcus spp.*
CRL course Copenhagen 20-22 April 2009

20th April (afternoon)

MIC on 5 *Staphylococcus* isolates including the QC reference strain:
1- strain 1
2- strain 2
3- strain 3
4- strain 4
QC strain :*S. aureus* ATCC 29213

Protocol:
MIC determination of *Staphylococcus*

1. Check the culture for contaminations

2. Calibrate the nephelometer with the McFarland standard:
   Turn it gently upside-down a couple of times until completely dissolved
   Do NOT shake or mix the standard

Do NOT touch the standard in the area that enters the nephelometer

3. Pick material from 3-4 colonies (to avoid only picking bacteria that lost their resistance) and prepare a suspension in 4 ml saline (use the inside of the tube) – vortex mix.
4. Adjust the suspension to McFarland 0.5 (add material or add saline) this corresponds to ~ 1-2 x 10^8 CFU/ml

5. Transfer 50 µl of the suspension to 10 ml of "MH broth".
Critical step for cross-contamination ! Use tips with extra length.

6. Exchange the screw cap with a dosing head – do NOT touch the dosing tip !

7. Turn upside down

8. Inoculate 50 µl per well in the DKMVR2 panels- Inoculum is now ≈5 x 10^5 CFU/ml.

9. Seal the panel with normal sealing – tighten all way around the edge to avoid evaporation.

10. Make purity control by spreading a loop (1 µl) of the final suspension on a blood agar plate

12. Incubate at 37°C for 18-20 hours.

To avoid growth of the inoculum, no more than 15-20 minutes should pass from suspensions are prepared to the inoculation and incubation occurs.
Incubate 18-20h at 36C