PROTOCOL FOR MIC-TESTING WITH SENSITITRE ON SALMONELLA AND CAMPYLOBACTER

CRL course Copenhagen March 2008

TUESDAY (MORNING)

MIC on 4 Campylobacter isolates including the QC referencestrain:

C-2-3
C-2.5
C-2.6
C. jejuni ATCC 33560

WEDNESDAY (AFTERNOON)

MIC on 2 salmonella isolates plus the QC referencestrain *E. coli* ATCC 25922:

S-2.2
S-2.5
E.coli ATCC 25922

THURSDAY (MORNING)

Reading of the MIC-results for all isolates: Campylobacter, Salmonella and QC referencestrains.

MIC ON CAMPYLOBACTER

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 ends up in the nephelometer

3. Pick material from 3-4 colonies (to avoid only picking bacteria that lost their resistance)

4. Dissolve completely in 4 ml saline (use the inside of the tube) – vortex mix.

5. Adjust to McFarland 0.5 suspension (add material or add saline) ~ 1-2 x 10^8 CFU/ml

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

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

8. Turn upside down

9. Inoculate **100 µl per well** in the **DKMVC3 panels** (the panel is for two isolates). Flow bench is recommended to minimize contaminations. Inoculum is now 50,000 CFU/well = 5 x 10^5 CFU/ml.

10. Seal the panel with the **perforated sealing** – tighten all way round the edge to avoid evaporation.

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

12. Incubate at 36˚C at **10% CO_2 for 48 hours**. Make sure that there is plenty of water in the tray in the bottom of the CO_2-incubator. Alternatively: Campy-bags can be used.

To avoid growth of the inoculum, **no more than 15-20 minutes** should pass from suspensions are prepared to the inoculation and incubation occurs.

Especially important for Campylobacter:

To achieve good growth it is important to:
- only use fresh cultures, directly from the incubator
- after storage in a freezer, cultures can be subcultivated one extra time before MIC-testing
- do not leave cultures on the table for longer periods, forexample in coffee breaks
- minimize the cultures stay in saline

MIC ON SALMONELLA

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 ends up in the nephelometer

3. Pick material from 3-4 colonies (to avoid only picking bacteria that lost their resistance)

4. Dissolve completely in 4 ml saline (use the inside of the tube) – vortex mix

5. Adjust to McFarland 0.5 suspension (add material or add saline) ~ 1-2 x 10^8 CFU/ml

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

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

8. Turn upside down

9. Inoculate **50 µl per well** in the **DKMVN4 panels**. Flow bench is recommended to minimize contaminations. Inoculum is now 5000 CFU/well = 1 x 10^5 CFU/ml.

10. Seal the panel with normal sealing – tighten all way round the edge to avoid evaporation.

11. 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.