VetMIC Brachy is an MIC-based (Minimal Inhibitory Concentration) broth dilution method for susceptibility testing of Brachyspira spp.

The antimicrobial agents are dried in serial twofold dilutions in tissue culture trays (48 wells/tray).

**Inoculation**

Each well is inoculated with 0.5 ml brain heart infusion (BHI) broth with 10% fetal calf serum and approx. 10⁶ CFU/ml of Brachyspira sp.

To get this inoculum density, two well-filled plastic loops (1µl) of bacterial material is suspended in 2 ml BHI broth. The bacteria are harvested from a three day old agar culture. This suspension contains about 10⁸ CFU/ml and is diluted 1:100 to get the final inoculum. Thus, to prepare 30 ml inoculum 300 µl of the suspension is added.

The inoculum density should be verified regularly by viable counts. A simplified method is to take 10 µl of the final inoculum and dilute in 10 ml BHI. From this dilution 100 µl is spread on an agar plate. This should give about 100 CFU.

**Incubation**

The panels are placed in an anaerobic atmosphere as soon as possible after inoculation. When stacking the panels, only the uppermost needs a lid. Never place more than four panels in a stack. Incubate on a shaker in 37°C for four days. For example, streak the bacteria on fresh agar on Monday, start the susceptibility test on Thursday and read the results following Monday. Thawed isolates should be subcultured at least twice before susceptibility testing.
### Reading results

The panel is placed on top of a viewing device, in form of a rack with an enlarging mirror. A bench lamp giving indirect light in a dark room facilitates reading. The control wells are checked for adequate growth. The MIC is read as the lowest concentration completely inhibiting visible growth. Every isolate should be checked for pure culture by phase contrast microscopy of material from the control well.

### Quality control

For laboratories performing MIC tests routinely, quality control organisms should be included at least once weekly and for each new batch of broth used. If the method is used infrequently, each test round should include a control organism. For these panels, *Brachyspira hyodysenteriae* ATCC 27164 is a suitable control strain.

### Shelf life and storage

Panels should be stored at room temperature. The shelf life is at least three years. Opened packages should be resealed and used within two weeks.

### References

No internationally recognised standards are established for susceptibility testing of *Brachyspira* so far. However, our experience is that VetMIC Brachy gives good and reproducible results for *Brachyspira hyodysenteriae* and *Brachyspira pilosicoli*.

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### Panel design VetMIC Brachy Art. No. E395107

Panel for antimicrobial susceptibility testing of *Brachyspira*

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Tia, Tiamulin; Val, Valnemulin; Dox, Doxycycline; Tlv, Tylvalosin; Lin, Lincomycin; Tyl, Tylosin; dist control, control with distilled water

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The method is used in SVARM, the Swedish Veterinary Antimicrobial Resistance Monitoring programme, National Veterinary Institute (SVA), Uppsala, Sweden, ISSN 1650-6332.

