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P0160 Development of a EUCAST disk diffusion method for rapidly growing anaerobic bacteria using fastidious anaerobe agar (FAA)

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Background: Resistance even to broad-spectrum antibiotics like meropenem and piperacillin-tazobactam is increasing in anaerobic bacteria, predominantly in the *Bacteroides fragilis* group. In order to detect resistance clinical laboratories today mainly use gradient tests. This method however is expensive and hampered by poor quality for certain antibiotics. With the increase in resistance there is an increasing demand for an inexpensive and reliable susceptibility testing method in anaerobic bacteria. We investigated the performance of FAA as a medium for a EUCAST disk diffusion method for rapidly growing anaerobic bacteria.

Materials/methods: Disk diffusion testing was performed with a McFarland 1 inoculum suspension on in-house produced FAA plates supplemented with 5% defibrinated horse blood. To make the method adaptable for as many laboratories as possible we investigated several parameters, including four different methods of anaerobic incubation (the anaerobic workstation (Ruskinn), gas-generating envelopes (Anaerogen and Gaspak), and the Anoxomat system), FAA powder from four different manufacturers (LabM, EOlabs, Bioconnections and Acumedia), different incubation temperatures (35 and 37°C) and different incubation intervals (16-24 h). These variables were thoroughly tested for six quality control strains: *Bacteroides fragilis* ATCC 25285, *Clostridium difficile* ATCC 700057, *Clostridium perfringens* CCUG 1795T, *Fusobacterium necrophorum* ATCC 25286, *Peptostreptococcus anaerobius* ATCC 27337 and *Prevotella melaninogenica* CCUG 4944 for clinically relevant antimicrobial agents of different classes. For each experiment zone diameters and the quality of growth were evaluated.

Results: Growth of the bacteria tested was confluent on FAA even after 16 h of incubation. Zone edges were sharp and easily readable. Variation of the incubation method or temperature resulted in minor differences in zone diameters (*figure 1)*. Using FAA from four manufacturers resulted in insignificant differences in zone sizes, with two clinically significant deviations only for Acumedia agar combined with metronidazole.

Conclusion: FAA is a reliable medium for disk diffusion of anaerobic bacteria when using agar from at least three manufacturers. Growth was confluent for all bacteria tested and there was little to no variation in zone sizes when changing the different variables included in this study. Implementing our method in clinical laboratories is straightforward with no necessary adaptations in laboratory instrumentation.

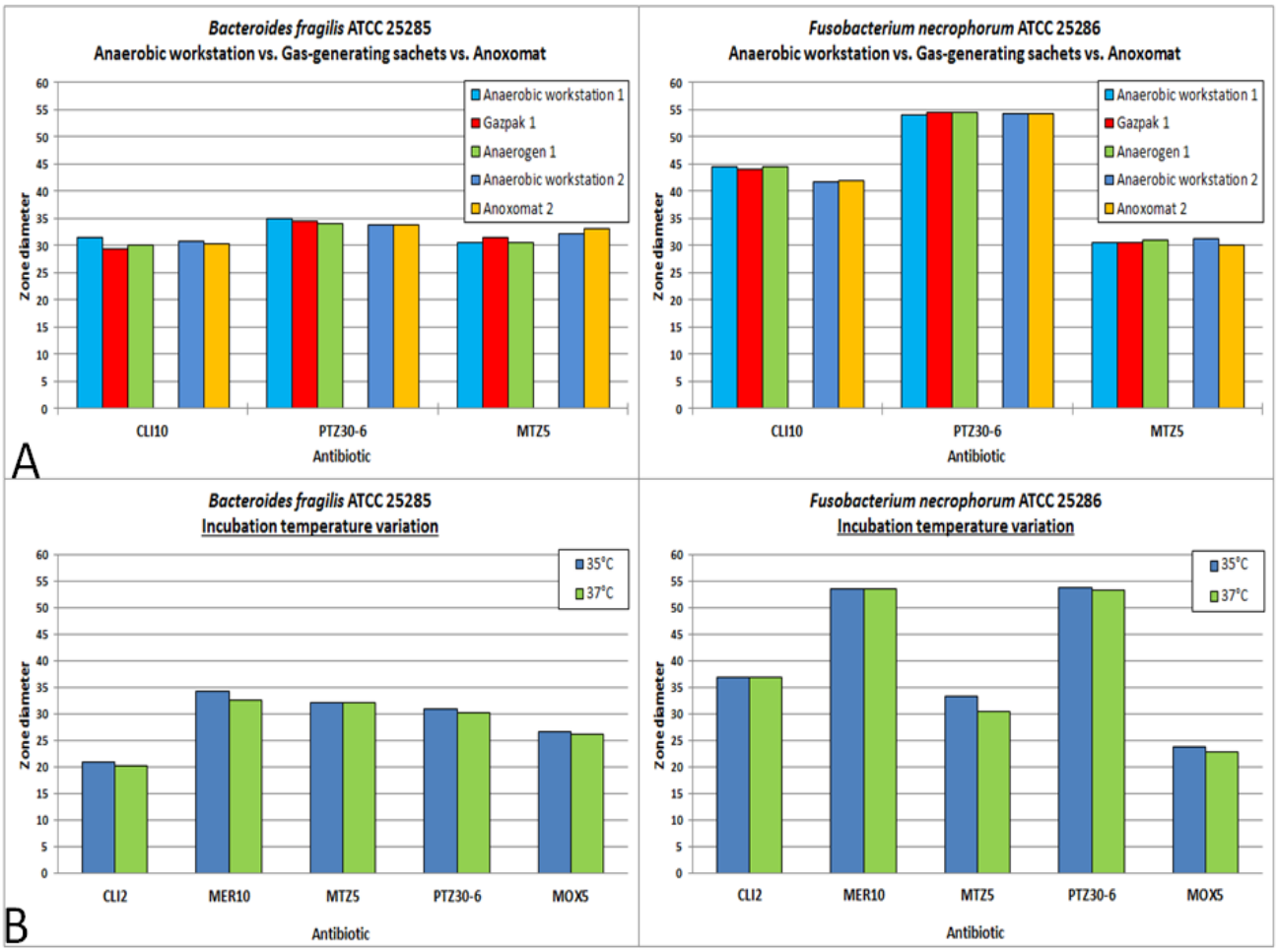

Figure 1. Inhibition zone diameters for *Bacteroides fragilis* ATCC 25285 and *Fusobacterium necrophorum* ATCC 25286 vs. a) anaerobic incubation method and b) incubation temperature. CLI = clindamycin, PTZ = piperacillin-tazobactam, MTZ = metronidazole, MER = meropenem, MOX = moxifloxacin. Number behind antibiotics indicate the used disk potency (µg). Anaerobic workstation, Gaspak and Anaerogen 1 is the first test. Anaerobic workstation and anoxomat 2 was tested separately. Zone diameter values are average values of 3 runs.