

P1-45

Enhanced growth of *Francisella tularensis* in a liquid nutrient medium (medium T)

D. Jacob¹, H. R. Nattermann¹, R. Krüger¹, S. Becker¹, R. Grunow¹

¹Robert Koch-Institute, Centre for Biological Safety (ZBS 2), Berlin, Germany

Aim: Enrichment and isolation of *Francisella tularensis* from environmental or clinical samples possibly containing disturbing flora.

Methods: The liquid nutrient medium T (Pavlovich and Mishar'kin, 1987) based on heart-brain infusion, bactotryptone, technical casamino acids and a salt-glucose-cysteine-solution was tested for confirmation of growth and enrichment of *F. tularensis*. For selective cultivation of *F. tularensis* from environmental samples or samples with high concentration of contaminating flora, antibiotics and a fungicide (colistin, trimethoprim, vancomycin, sulfamethoxazol, amphotericin B) were added to the medium (modified medium T). The cell amount of *F. tularensis* was determined over four days on cysteine heart blood agar.

Results: Enhanced growth of *F. tularensis* strain LVS could be shown by inoculating a defined amount of the bacteria (2×10^2 cfu/ml) into medium T. After three days of incubation, a growth up to 5×10^8 cfu/ml could be detected independently from the addition of antibiotics to the media. Growth of other highly pathogenic bacteria like *Bacillus anthracis*, *Yersinia pestis*, *Burkholderia mallei* and *Burkholderia pseudomallei* could also be demonstrated in medium T. On the other hand, it could be shown that the modified medium T inhibited the growth of selected adulterating bacteria like *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Yersinia pseudotuberculosis*. The application of modified medium T for the analysis of organ samples from foxes for an epidemiological investigation on distribution of *F. tularensis* in the German federal state of Brandenburg confirmed an inhibition of contaminating flora.

Conclusions: Cultivation of the fastidious bacteria *F. tularensis* is known to be difficult and sometimes the recovery of the bacteria from samples with high concentration of contaminating flora is a diagnostic challenge. Therefore, the nutrient medium T can be highly recommended for the cultivation of *F. tularensis* from environmental or clinical samples and also for other highly pathogenic bacteria.