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A COMPARISON OF THREE METHODS FOR ISOLATION OF *SALMONELLA* FROM BIOLOGICAL WASTE

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Material and metod

A total of 270 samples of sludge, soil, and liquid manure were artificially contaminated with *Salmonella senftenberg* H_2S^+at target levels of 10^1-, 10^2- and 10^3 colony-forming units (CFU)/g.

The method 1 is a presence/absence method including recovery of sub-lethally damaged *Salmonella* spp. designed to process samples of up to 50 g wet weight. This method consisted of pre-enrichment in buffered peptone water with addition of novobiocin at 36 ± 2°C (20 ± 2 h incubation), selective motility enrichment in Rappaport Vassiliadis broth (36 ± 2°C and 42 ± 1°C for 20 ± 2 h incubation) which inhibits the growth of other micro-organisms but promotes that of *Salmonellae*, preparation of pure cultures by inoculating special solid media (BPLS- und XLD agar) with subcultures (Nutrient agar) and biochemical (API 20E) and serological identification (0 and H agglutination).

The method 2 (MPN-technique) requires following stages: suspension of the sample (20 g) in 0,9% m/V sodium chloride, serial dilutions of this suspension in the same dilutet, transfer of 1 ml of these diluted suspensions into 3 tubes containing 9 ml peptone water (36 ± 2°C for 20 ± 2 h incubation) followed by selective enrichment in Rappaport Vassiliadis broth for 20 ± 2 h at 42 ± 1°C and isolation on BPLS and XLD plate.

The method 3 specified a membrane filtration procedure for the quantitative recovery and enumeration, by enrichment in tetrathionate brilliant green broth, and culture of individual colonies on Rambach agar at 37°C for 24 h. Principle: The homogenized diluted sample (25 g in 225 ml PBS) is centrifuged and filtered, the membrane filter recovered aseptically and incubated at 36 ± 2°C on a sterile glass fibre disk soaked with resuscitation medium (Tetrathionate broth). After 24 h membrane is recovered aseptically and incubated at 36 ± 2°C on chromogenic medium (Rambach agar). The membrane is examined after 24 h

and positive colonies are quantified .The presence of *Salmonella* spp. is indicated by bright red colonies resulting from fermentation of propylene glycol. To distinguish *Salmonella* spp. from occasional *Citrobacter* spp., spray an aerosolised solution of 4-methylumbelliferyl caprylate in ethanol directly onto Rambach agar. The presence of *Salmonella* spp. is indicated by fluorescence of the colonies under UV light. Calculation of the number of *Salmonellae* (present per gram wet weight of the original sample) is by multiplying the number of fluorescent colonies on the filter. Number present per g wet weight are calculated according to

$$c = \frac{an}{bd}$$ ***were***

c is the original concentration of *Salmonellae*

a is volume filtered through each membrane (10 ml)

b is initial dilution factor for the sample in PBS (10 ml)

d is dilution factor for the serial dilution in water (10^{o} to 10^{-3})

n is the count of *Salmonella* colonies on the membrane

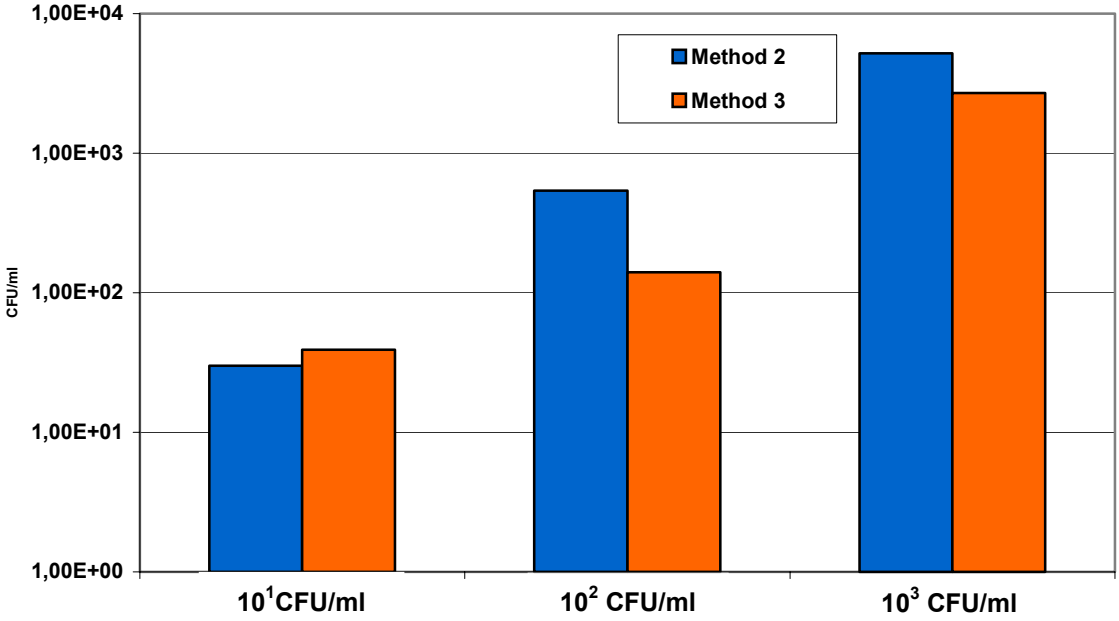
Results and discussion

All three *Salmonella*-concentrations used could be detected again by the methods 2 and 3 in all type of biological waste. The qualitative detection method (method 1) was also able to detect *Salmonella senftenberg* in all examined samples at 37° and 43°C.

These results demonstrate that it is easy to obtain reproducible results with the three methods used, and that they can be recommended for the detection of *Salmonellae* from all types of biological waste. To what extent these tested methods are effective with substrates, which are not artificially contaminated, could not be examined in this work. Further investigations with different naturally contaminated substrates are necessary.

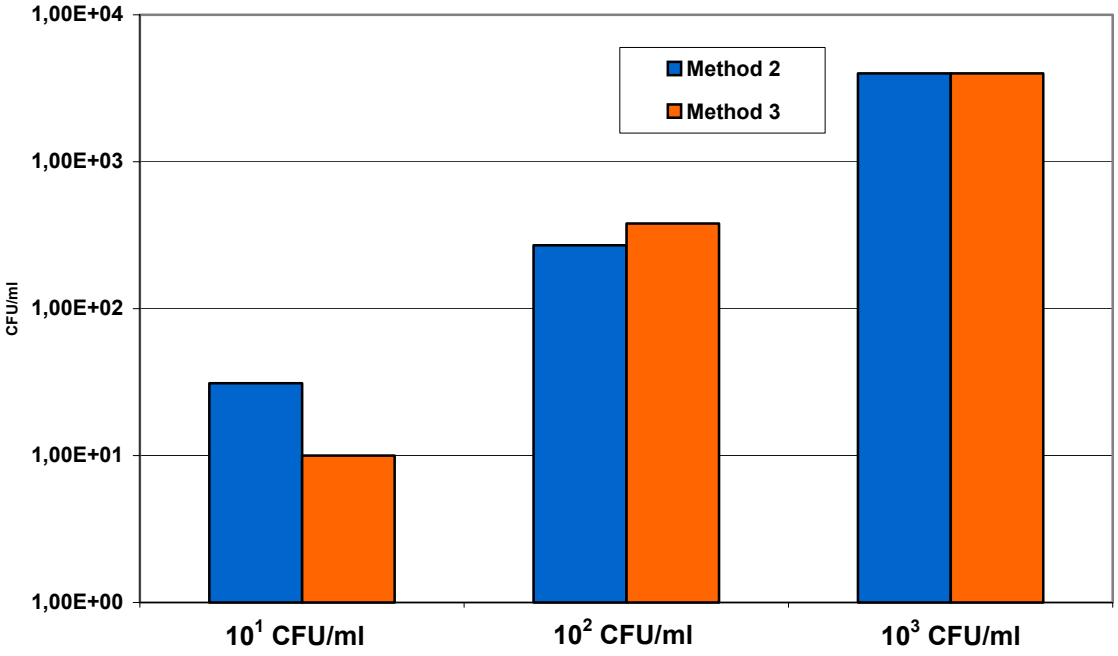
For the following diagram calculated mean values were used.

Fig. 1: Calculated mean values of the examined soil samples



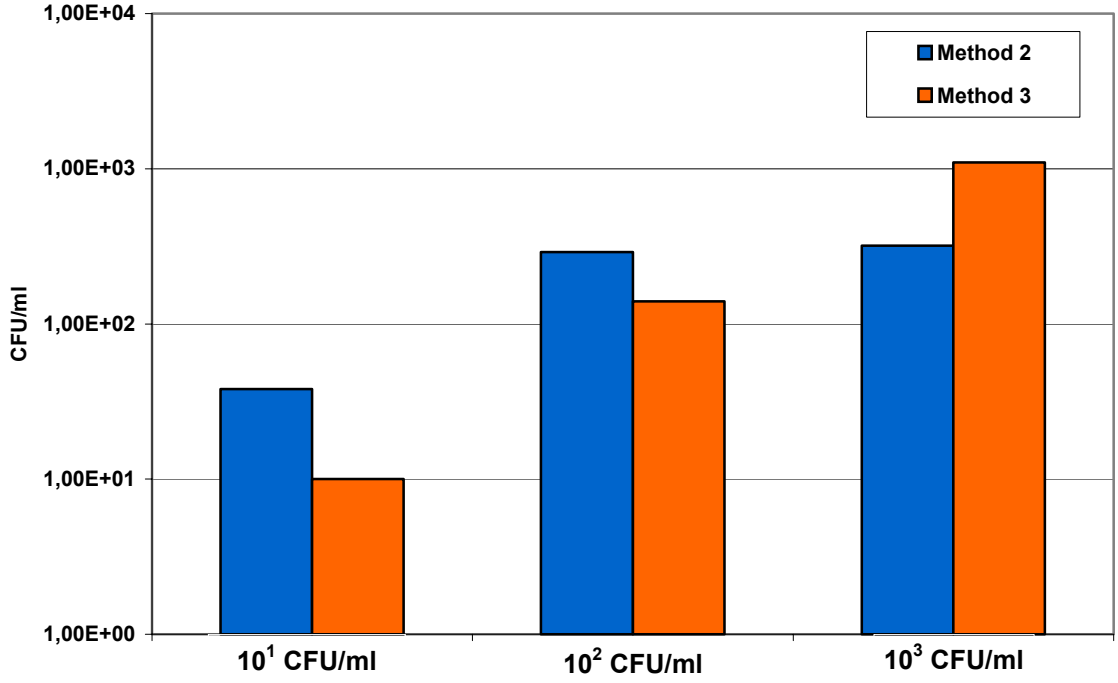
CFU: colony forming units

Fig. 2: Calculated mean values of the examined liquid manure samples



CFU: colony forming units

Fig. 3: Calculated mean values of the examined sludge samples



CFU: colony forming units

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2. *Anonym (2005): Detection and enumeration of Salmonella spp. in sludges, soils, soil improvers, growing media and bio-wastes . Part 1: Membrane filtration method for quantitative resuscitation of sub-lethally stressed bacteria (to confirm efficacy of log drop treatment procedures)*