

Preliminary evaluation of in-house miniaturised biochemical test strips for identification of common enteric Gram-negative pathogens encountered in veterinary clinical practice

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Introduction and Objectives: Microorganisms are routinely identified using biochemical tests. Consequently, miniaturisation of biochemical reactions makes identification more economical. In this study, citrate, urease and triple sugar ion (TSI) tests were miniaturised for the identification of selected bacterial isolates encountered in veterinary clinical practice .

Methods: Citrate and urease tests were conducted using 4 mL (Standard), 0.40 mL and 0.20 mL (strips). For TSI, 5 mL (standard), 0.50 mL and 0.30 mL (strips) volumes were tested. Media from the same batch were used in all experiments. Bacterial isolates were selected to test both positive and negative phenotypic observations. *Klebsiella* spp. was selected to observe citrate and urease test positive colour changes and TSI positive gas formation. *E. coli* was selected to observe citrate, and urease test negative colour changes and TSI positive gas formation. *Salmonella* spp. was selected to observe TSI positive colour changes and H₂S formation. The same bacterial suspension was used to inoculate each set of standard and miniaturised test preparations. All experiments were incubated aerobically at 37 °C. All tests were conducted in three replicates. Observations taken by five independent observers were analysed with one way ANOVA. Results were also compared using an artificial intelligence aided color-coding system.

Results: For all three organisms, citrate and urease tests produced expected colour changes in all miniaturised volumes without any significant difference compared to standards. For the TSI test, the gas production was not accurately observable for *E. coli* and *Klebsiella* spp. beyond 0.50 mL, though the colour changes in the media were not significantly different compared to standards. H₂S gas production was observable, but the colour changes were significantly different in the TSI test for *Salmonella* spp. beyond 0.50 mL miniaturisation.

Conclusion: These preliminary findings show that in both citrate and urease tests, the miniaturising was successful up to 0.20 mL with a 95% cost reduction for identification of *E. coli*, *Salmonella* spp., and *Klebsiella* spp. However, the TSI test was successfully miniaturised only up to 0.50 mL with 90% cost reduction for these three isolates. Most importantly, results in 0.20 mL test strips were obtained within 6 hours. These findings need to be evaluated among a wider group of known isolates as well as clinical isolates.

Keywords: Miniaturizing, urease, citrate, triple sugar ion, food pathogens

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