ABSTRACT

The mastitis causing microorganisms resist beta-lactam antibiotics by releasing beta-lactamase and the enzyme can be traced in raw milk. This study was aimed at developing a novel platform test to detect beta-lactam antibiotics residues in raw milk based on the Hardy Diagnostic Beta-lactamase Test (HDBT) reagent. The HDBT ingredients modified were penicillin, sodium chloride, trisodium citric acid, trisodium phosphate and phenol red dissolved in distilled water. Pooled raw milk samples were obtained from 3 Friesians and 3 Ayrshires lactating cows identified to have subclinical mastitis and treated using beta-lactam antibiotics. The appropriate mixing ratios were investigated at nine levels. Investigation on the effect of breeds on the test method results was also carried out. Evaluations to determine the colour differences between beta-lactam positive and negative raw milk samples for all the experiments were carried out using trained panelists. The results indicate that gradual addition of trisodium phosphate and phenol red in the reagent showed significant difference ($P \leq 0.05$) between a beta-lactam positive and negative raw milk sample. Ratio 5:5 was selected as the best and had significant difference ($P \leq 0.05$) from the others. Conversely, the test method indicated no significant difference ($P \leq 0.05$) between the Friesians and Ayrshires raw milk samples. This method can be used along the raw milk collection routes to accept, set aside or reject raw milk suspected to have residues. The colour observed for a beta-lactam negative sample was fuchsia purple while peach or pink signified a positive sample.

Key words: Raw milk, antibiotic residues, beta-lactam, beta-lactamase enzyme, trisodium phosphate