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## Development of enzyme substrate assay for monitoring *E. coli* species in milk and milk products

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In order to meet the emerging demand of dairy and water industry, an attempt was made to develop a rapid enzyme substrate assay its detection in milk and milk products, with working based on the principle of targeting “enzyme-substrate reactions” for specific marker enzyme (s) of target bacteria releasing free chromogen which can be visually detected by color change, after initial enrichment of the test organism in novel selective medium. Initially, growth pattern of *E. coli* was studied in five different commercial media wherein Tergitol-7 proved to be more selective and inhibitory for potential contaminants commonly encountered as background micro flora and demonstrated higher log counts reduction in comparison with EMB, VRBA, Difco MI and MacConkey broth. Tergitol-7 broth was finally selected and its growth supplements namely peptone, yeast extract, lactose and potassium chloride were optimized based on log counts study. It was supplemented / or modified with new selective agents to have higher selectivity towards background micro-flora and better sensitivity for the target organism namely *E. coli*. The newly developed medium i.e. EC-SM was established as selective medium for the growth of *E. coli* and subsequently, was transformed into an assay for detection of *E. coli* using different novel marker enzymes. The “enzyme assay” gives confirmatory detection of *E. coli* based on green color appearance in the medium during selective enrichment of the test organism. The developed assay was further, validated in house using spiked raw milk as well as IS-5887 (Part-I) 1976 method (reaffirmed 2009) adopted for testing of *E. coli*. The assay was evaluated under field conditions with raw milk, pasteurized milk and ice-cream samples procured from different sources.

### Biography

Ramakant Lawaniya is currently working as a Research Associate at National Referral Centre for Milk Quality and Safety, in the department of Microbiology, National Dairy Research Institute, India on “Development of Biosensor and Micro techniques for detection of Pesticide Residues, Aflatoxins, Heavy Metals and Bacterial Contaminants in milk”. He obtained his PhD from the National Dairy Research Institute, India, in 2014 researching “Development of enzyme substrate assay for monitoring *E. coli* in milk and milk products”. Indian Patent filing is in progress (Lawaniya et. al.) under the supervision of Naresh Kumar, Principal Scientist. His PhD work was supported by the esteemed Rajeev Gandhi Fellowship, University Grant Commission, New Delhi, Government of India (2010-2015) and the Institutional Fellowship (2008-2011) National Dairy Research Institute, Indian Council of Agriculture Research, India.

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