Australian Standard™

Water microbiology

Method 21: Examination for coliforms and *Escherichia coli*—Determination of most probable number (MPN) using enzyme hydrolysable substrates

PREFACE

This Standard was prepared by the Joint Standards Australia/New Zealand Committee FT-020, Water Microbiology, as part of a series of methods for the microbiological examination of waters for domestic and industrial use.

This Standard was prepared by the Australian members of the Joint Standards Australia/Standards New Zealand Committee FT-020. After consultation with stakeholders in both countries, Standards Australia and Standards New Zealand decided to develop this Standard as an Australian Standard rather than an Australian/New Zealand Standard.

The objective of preparing this Standard is to establish the principles of determining the most probable number (MPN) using enzyme hydrolysable substrates.

The laboratory should have a clearly defined quality control system to ensure that the apparatus, culture media, reagents and technique are suitable for the test. The use of positive controls is part of this system.

The term 'informative' has been used in this Standard to define the application of the appendix to which it applies. An 'informative' appendix is only for information and guidance.

FOREWORD

The numbers of coliforms and *Escherichia coli* present in the water sample are simultaneously estimated using a multiple tube dilution or multiple well technique.

The medium contains chromogenic and fluorogenic substrates targeted by coliforms and *E. coli* with the pH and chemical environment being optimized for their growth. Because ammonium sulphate is the only source of nitrogen, the metabolic activity of the target microorganisms is directed towards the substrates, so that non-target microorganisms are inhibited.

Coliforms (including *E. coli*) will metabolize the chromogenic substrate ortho-nitrophenyl-β-D-galactopyranoside (ONPG) using the enzyme β-galactosidase to produce a yellow (positive) colouration. *E. coli* will also metabolize the fluorogenic substrate 4-methyl umbelliferyl-β-D-glucuronide (MUG) using the enzyme β–D-glucuronidase to produce a fluorogen that fluoresces under long wave ultraviolet light. Note that some strains of coliforms/*E. coli* detected and enumerated by this method may not be detected by traditional methods. There are strains of *E. coli,* including strains of public health significance (for example, *E. coli* O157), that may not be detected by this method.