

ISO 6579-4:2025-01 (E)

Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 4: Identification of monophasic *Salmonella* Typhimurium (1,4,[5],12:i:-) by polymerase chain reaction (PCR)

| Contents | Page |
|---|------|
| Foreword..... | iv |
| Introduction..... | v |
| 1 Scope..... | 1 |
| 2 Normative references..... | 2 |
| 3 Terms and definitions..... | 2 |
| 4 Principle..... | 2 |
| 4.1 General..... | 2 |
| 4.2 Preparation of well-isolated colonies..... | 2 |
| 4.3 Suspension of a colony..... | 2 |
| 4.4 Amplification and detection..... | 3 |
| 5 Culture media and reagents..... | 3 |
| 6 Equipment and consumables..... | 3 |
| 7 Presumptive monophasic <i>Salmonella</i> Typhimurium..... | 4 |
| 8 Culturing the isolate..... | 4 |
| 9 Procedure..... | 4 |
| 9.1 Preparation of cell suspension or DNA..... | 4 |
| 9.2 PCR amplification and detection..... | 4 |
| 9.2.1 General..... | 4 |
| 9.2.2 PCR controls..... | 4 |
| 10 Expression of results..... | 4 |
| 11 Performance characteristics of the method..... | 5 |
| 11.1 Validation in accordance with ISO 17468..... | 5 |
| 11.2 Performance characteristics..... | 5 |
| 11.2.1 Method(s) evaluation study..... | 5 |
| 11.2.2 Interlaboratory study..... | 5 |
| 12 Test report..... | 6 |
| 13 Quality assurance..... | 6 |
| Annex A (normative) Culture media and reagents..... | 7 |
| Annex B (informative) Probe-based multiplex real-time PCR assay for the identification of monophasic <i>Salmonella</i> Typhimurium (1,4,[5],12:i:-)..... | 9 |
| Annex C (informative) Agarose gel-based multiplex target PCR assay for the identification of monophasic <i>Salmonella</i> Typhimurium (1,4,[5],12:i:-)..... | 13 |
| Annex D (informative) Agarose gel-based single target PCR assay for the identification of monophasic <i>Salmonella</i> Typhimurium (1,4,[5],12:i:-)..... | 18 |
| Annex E (informative) Performance characteristics..... | 25 |
| Bibliography..... | 32 |