# ISO 8199:2018 (E)

## Water quality — General requirements and guidance for microbiological examinations by culture

# Contents


Foreword

Introduction

1 Scope

2 Normative references

3 Terms and definitions

4 Principle

5 General measurement requirements

5.1 Uniformity of temperatures
5.2 Incubation times
5.3 Volumes and masses

6 Diluents and culture media

6.1 General
6.2 Quality requirements of ingredients
6.3 Diluents

7 Sterilization and decontamination

7.1 Sterilization of apparatus and glassware
7.2 Sterilization of consumables
7.3 Decontamination of glassware and materials after use
7.4 Waste management

8 Samples and sample handling

8.1 Sampling
8.2 Sample preparation
8.2.1 Waters and other aqueous matrices
8.2.2 Swabs
8.2.2.1 General
8.2.2.2 Stick swab preparation
8.2.2.3 Sponge swab preparation

9 Enumeration (quantitative) methods

9.1 Inoculation of test portions in (or on) solid media
9.1.1 General
9.1.2 Pour plate technique
9.1.2.1 General
9.1.2.2 Test portion
9.1.2.3 Inoculation
9.1.3 Spread plate technique
9.1.3.1 General
9.1.3.2 Test portion
9.1.3.3 Inoculation
9.1.4 Membrane filtration technique
9.1.4.1 General
9.1.4.2 Test portion
9.1.4.3 Filtration apparatus
9.1.4.4 Filtration

9.1.4.5 Transfer of membrane filter
9.1.4.6 Membrane transfer techniques using liquid media or diluents
9.1.5 Incubation
9.1.6 Counting and confirmation from solid media
9.1.6.1 General
9.1.6.2 Colonies to be counted and confirmed
9.1.7 General guidance for calculation of results
9.1.7.1 General
9.1.7.2 General case
9.1.7.3 Case with confirmation
9.1.8 Expression of results
9.1.8.1 General
9.1.8.2 Method of calculation: General case (counting of total colonies or target colonies)
9.1.8.3 Method of calculation: General case with confirmation
9.1.8.4 Method of calculation: Low counts
9.1.8.4.1 Case when one Petri dish contains fewer than 10 colonies
9.1.8.4.2 Case when the Petri dish contains no colonies
9.1.8.5 Special cases
9.1.8.5.1 General
9.1.8.5.2 Case 1: High background growth with target colonies
9.1.8.5.3 Case 2: High background growth without target colonies
9.1.8.6 Methods of calculation: unusual, estimated and unacceptable counts
9.1.8.6.1 General
9.1.8.6.2 Unexpected ratios when dilutions are used
9.1.8.6.3 All dishes are above the upper counting limits
9.1.8.6.4 Only the dish with the highest dilution is countable
9.1.8.7 Uncertainty of test results
9.2 Enumeration using a liquid medium
9.2.1 General
9.2.2 Procedure
9.2.3 Choice of inoculation system
9.2.3.1 General
9.2.3.2 Single-dilution system
9.2.3.3 Multiple-dilution system
9.2.3.3.1 General
9.2.3.3.2 Symmetric dilution system
9.2.3.3.3 Non-symmetric dilution system
9.2.4 Incubation
9.2.5 Interpretation of results
9.2.6 Uncertainty of test results
9.2.7 Determination of MPN values
9.2.7.1 General
9.2.7.2 Mathematical formulae
9.2.7.2.1 Formula for one series of tubes
9.2.7.2.2 Precision estimates for single-dilution assays
9.2.7.2.3 Precision estimates for symmetrical multiple-dilution assays
9.2.7.3 MPN tables
9.2.7.3.1 Tables for single-dilution systems
9.2.7.3.2 Tables for multiple-dilution systems: Three successive dilutions
9.2.7.4 Determination of MPN values using an MPN calculator

10 Detection (qualitative) methods

10.1 General
10.2 Procedure
10.3 Uncertainty of test results

11 Performance characteristics of methods

12 Analytical quality control

12.1 General
12.2 Internal quality control
12.2.1 General
12.2.2 Process controls
12.2.2.1 General

12.2.2.2 Replicate testing
12.2.2.3 Spiked samples
12.2.2.4 Microorganisms for internal quality control
12.2.2.5 Assessing internal quality control results
12.3 External quality assessment

Annex A (informative) Criteria for the choice of technique

A.1 General
A.2 Factors concerning the quality of the result
A.2.1 General
A.2.2 Uncertainty of test results
A.2.3 Accuracy
A.2.3.1 Principle
A.2.3.2 Trueness
A.2.3.2.1 General
A.2.3.2.2 Quantitative errors
A.2.3.2.3 Qualitative errors
A.2.3.3 Precision
A.2.4 Detection level
A.2.4.1 Principle
A.2.4.2 Detection level of colony count procedures
A.2.4.3 Detection level with MPN procedures
A.2.5 Effects of membrane filter type and batches
A.3 Requirements concerning the nature of the sample
A.3.1 Nature of the microorganisms
A.3.2 Constituents of water samples

Annex B (informative) Confidence intervals for colony count technique and choice of method of calculation in special cases

B.1 Confidence intervals for colony count technique
B.2 Choice of the method of calculation in special cases, with one dish per dilution
B.2.1 Special cases not only with low numbers
B.2.2 Special cases with low numbers

Annex C (normative) Counting and calculations with two Petri dishes per dilution

C.1 Counting of colonies
C.2 Method of calculation: General case (counting of total colonies or target colonies)
C.3 Method of calculation: Case after confirmation
C.4 Method of calculation: Estimated counts
C.4.1 Case of two dishes (test sample or initial suspension or first dilution) containing fewer than 10 colonies
C.4.2 Case of two dishes (test sample or initial suspension or first dilution) containing no colonies
C.5 Special cases (counting of target or presumptive colonies)
C.6 Method of calculation: Special cases

Annex D (normative) Composition, preparation and performance testing of diluents

D.1 General
D.2 Composition and preparation
D.2.1 Saline solution
D.2.1.1 Composition
D.2.1.2 Preparation
D.2.2 Peptone diluent
D.2.2.1 Composition
D.2.2.2 Preparation
D.2.3 Peptone saline solution [maximum recovery diluent (MRD)]
D.2.3.1 Composition
D.2.3.2 Preparation
D.2.4 Quarter-strength Ringer's solution
D.2.4.1 Composition
D.2.4.2 Preparation
D.2.5 Phosphate buffer solution
D.2.5.1 Phosphate solution
D.2.5.1.1 Composition

**D.2.5.1.2 Preparation**
**D.2.5.2 Magnesium chloride solution**
**D.2.5.2.1 Composition**
**D.2.5.2.2 Preparation**
**D.2.5.3 Final solution**
**D.2.5.3.1 Composition**
**D.2.5.3.2 Preparation**
**D.3 Sterilization and storage**
**D.4 Performance testing**

**Page count: 56**