International Standard

ISO 13647

Water quality — Enumeration of culturable microorganisms — Colony count by spread plate inoculation on R2A medium

Qualité de l'eau — Dénombrement des micro-organismes revivifiables — Comptage des colonies par ensemencement par étalement sur un milieu gélosé R2A

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ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

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Foreword

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This document was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 4, *Microbiological methods*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 230, *Water analysis,* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

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Introduction

Treated drinking water contains a variety of microorganisms derived from various sources, such as raw water resource, different treatment steps within drinking water production and drinking water distribution networks.

Enumeration of the overall colony count of culturable microorganisms provides useful information for the assessment and surveillance of water quality. Colony counts are useful for assessing the raw water quality and efficiency of water treatment processes and provide an indication of the cleanliness and integrity of the drinking water distribution systems.

However, the main value of colony counts lies in the detection of changes from the expected values, based on representative monitoring. The change in colony counts can be caused for various reasons like biofilm formation, increase in water temperature, presence of microbially available nutrients or decrease in chlorine concentration. Any substantial increase in count can also be either a warning of potential treatment failure or contamination.

Nutrient rich tryptone yeast extract agar medium has been used for long time in water microbiology. ISO 6222 provides a monitoring method for heterotrophic plate counts in tap water quality verification. However, ISO 6222 only applies to the detection of microorganisms in nutrient rich conditions at 22 °C in 3 d or at 36 °C in 2 d.

Reasoner's 2 agar (R2A) medium, which has a low-nutrient composition and long incubation time (7 d at 22 °C), enables the determination of micro-organisms forming colonies after a longer incubation time. Furthermore, the low level of nutrients in the medium minimizes the colony size and overgrowth. Therefore, the R2A method is especially suitable for providing more information on the enumeration of heterotrophic colony counts in water with low colony counts.

The method is well suited for monitoring water for human consumption which is low in nutrients and is distributed in temperatures below 20 °C (see Reference [1]), for example, to measure the operational efficiency of the treatment process of public drinking water supplies. The low-nutrient agar specified in this document for enumeration of culturable microorganisms usually gives higher colony counts from water samples than typically used nutrient-rich formulations of culture media (see References [2], [3] and [4]).

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Water quality — Enumeration of culturable microorganisms — Colony count by spread plate inoculation on R2A medium

WARNING — Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices.

IMPORTANT — The tests in this document shall be carried out by suitably qualified staff.

1 Scope

This document specifies a method for the enumeration of culturable microorganisms in water by counting colonies on a low-nutrient agar culture medium by spread plate inoculation after incubation at 22 °C for 7 d.

This document is applicable to:

— water from the treatment process of public drinking water supplies (e.g. process water in the waterworks);

— chlorinated and non-chlorinated tap water in distribution systems and containers;

— bottled water;

— well water intended for human consumption.

For detailed information on the performance characteristics, see Annex B.

This document can be applied to other water matrices if the appropriate validation of performance of this method has been undertaken by the laboratory prior to use.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 5667-5, *Water quality — Sampling — Part 5: Guidance on sampling of drinking water from treatment works and piped distribution systems*

ISO 8199:2018, *Water quality — General requirements and guidance for microbiological examinations by culture*

ISO 11133, *Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media*

ISO 19458, *Water quality — Sampling for microbiological analysis*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

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