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Microbiological Performance Testing of Membrane Filters in Accordance with EN ISO 7704:2023

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Abstract

The requirements for the performance testing of membrane filters used for water quality testing by retention of microorganisms followed by direct enumeration on culture media are specified by ISO 7704. In its revised form, EN ISO 7704:2023 mandates that the suitability of the whole system, the membrane filter in combination with the solid culture medium, must be demonstrated for each batch. Apart from the manufacturers, microbiological laboratories using membrane filters in combination with culture media for water testing are obligated to perform the mandatory test modules. In this study, the release of membrane filters according to the revised EN ISO 7704:2023 is demonstrated using the specific standard EN ISO 7899-2 as an example. The tested Sartorius high flow membrane filters comply with the new EN ISO 7704:2023.

Introduction

The quantitative determination of microorganisms in water samples is a mandatory element of microbiological quality control. It applies to various types of water samples, including those with expected low numbers of microorganisms, such as drinking water or bottled water, as well as those with expected higher numbers of microorganisms, such as surface water or process water. Membrane filtration has been established as the regulatory-preferred method for microbial enumeration of water samples. In this regard, EN ISO 7704:2023[1] specifies the performance testing requirements for membrane filters used for the retention followed by direct enumeration of microorganisms. The performance testing also applies to specific standards such as ISO 7899-2[2], ISO 6461-2, ISO 9308-1, ISO 11731, ISO 14189 and ISO 16266. EN ISO 7704:2023 comprises different mandatory batch testing modules as well as supplementary quantitative testing modules (Figure 1).

The mandatory batch testing, consisting of modules 1 and 2, requires the simultaneous testing of units from a batch of membrane filters as well as units from a batch of a specific selective culture medium intended for use. This testing must be performed by manufacturers and microbiological laboratories for each batch of membrane filters with each batch of culture medium. Module 1 aims to determine the reference count, which is the total number of colonies on a non-selective reference medium obtained without the use of a membrane filter. Module 2, on the other hand, aims to determine the number of colonies on a membrane filter when using the specific selective culture medium. Additionally, if applicable, qualitative selectivity and specificity tests are conducted in module 2. By analyzing the results obtained in modules 1 and 2, it is possible to calculate the productivity ratio (P_R) of the entire system-, that is, the performance of the membrane filter in conjunction with the specific selective culture medium. This study demonstrates the batch release of cellulose nitrate membrane filters in accordance with the mandatory modules 1 and 2 of the revised EN ISO 7704:2023, utilizing the specific standard EN ISO 7899-2 as an example. EN ISO 7899-2 outlines a method for the detection and enumeration of intestinal enterococci in water by membrane filtration.

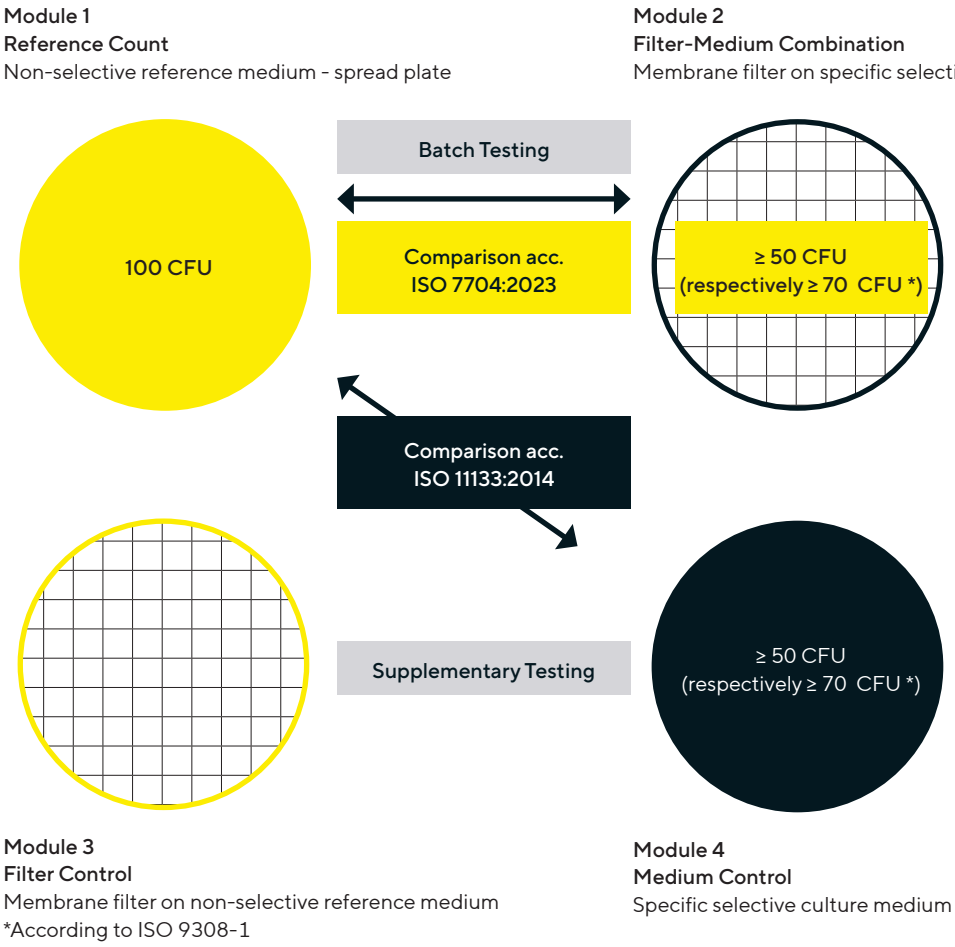

Figure 1: Schematic mandatory batch testing procedures (modules 1 and 2) and supplementary quantitative testing procedures (modules 3 and 4) according to new EN ISO 7704:2023. Taken from Sartorius Best Practice Guide[3].

Materials and Methods

For preparation and storage of culture media and diluents, the procedures have been adapted to the corresponding specific standards EN ISO 11133[4] or EN ISO 8199[5]. Each experiment was performed with three different lots of membrane filters and media in six replicates.

Product	Order No.
3 branch Microsart® Manifold	168M3-SS100
Microsart® e. jet pump	166MP-4
Cellulose Nitrate High Flow membrane filter (single-packed)	114H6--47----ACN
Cellulose Nitrate High Flow membrane filter (dispenser-ready)	114H6Z-47----SCM
Sartorius Tryptic Soy Agar (TSA)	14171----------A
BD Tryptic Soy Agar (TSA) plates	257632
Merck Slanetz & Bartley (S&B)	1.05262.0500

Table 1: Consumables, media, and equipment used in the study.

Tryptic soy agar (TSA) was used as a non-selective reference agar and Slanetz & Bartley (S&B) agar was chosen as a specific selective agar for recovery of intestinal enterococci (Table 1). All certified test microorganisms (Table 2) were diluted in sterile phosphate buffer (PBS) and incubated at 36 °C ± 2 °C for 44 h ± 4 h according to EN ISO 7899-2.

Test microorganisms	Function
Enterococcus faecalis WDCM 00009	Productivity
Enterococcus faecium WDCM 00177	Productivity
Escherichia coli WDCM 00013	Selectivity
Staphylococcus aureus WDCM 00034	Selectivity

Table 2: Certified test microorganisms used in the study.

Filtration

To determine the reference count, an inoculum containing 50 - 150 CFU of certified reference material, either *Enterococcus faecium* WDCM 00177 or *Enterococcus faecalis* WDCM 00009, was inoculated onto TSA using the spread plate technique in accordance with EN ISO 7704:2023. To assess the productivity rate, a membrane filter was placed on the manifold, a sterile funnel was mounted, and 10 mL of sterile PBS was poured into the funnel. Subsequently, the inoculum containing 50 - 150 CFU of either *E. faecium* WDCM 00177 or *E. faecalis* WDCM 00009 (using the same volume from the reference stock as for the reference count determination) was added. An additional volume of 10 mL of sterile PBS was added to disperse the inoculum before performing the filtration.

The membrane filters were then transferred onto the specific selective S&B agar and incubated as prescribed. For selectivity testing, 10^4 CFU of *Escherichia coli* WDCM 00013 and *Staphylococcus aureus* WDCM 00034 were each tested by membrane filtration and subsequently inoculated onto S&B agar.

$P_R = \frac{Ns}{No}$	**Ns** is defined by the total count of colonies obtained from membrane filters in intended use with the specific culture medium (S&B) according to module 2.
	No is defined by the total count of colonies obtained from plates of non-selective reference culture medium (TSA) by direct inoculation without a membrane filter according to module 1.

Table 3: Calculation of the productivity ratio.

Results

Colonies of *E. faecium* WDCM 00177 grown on S&B agar exhibit a light red to maroon coloration on the membrane filter (see Figure 2). *E. faecalis* WDCM 00009 colonies feature a red to maroon coloration on membrane filters when grown on S&B agar.

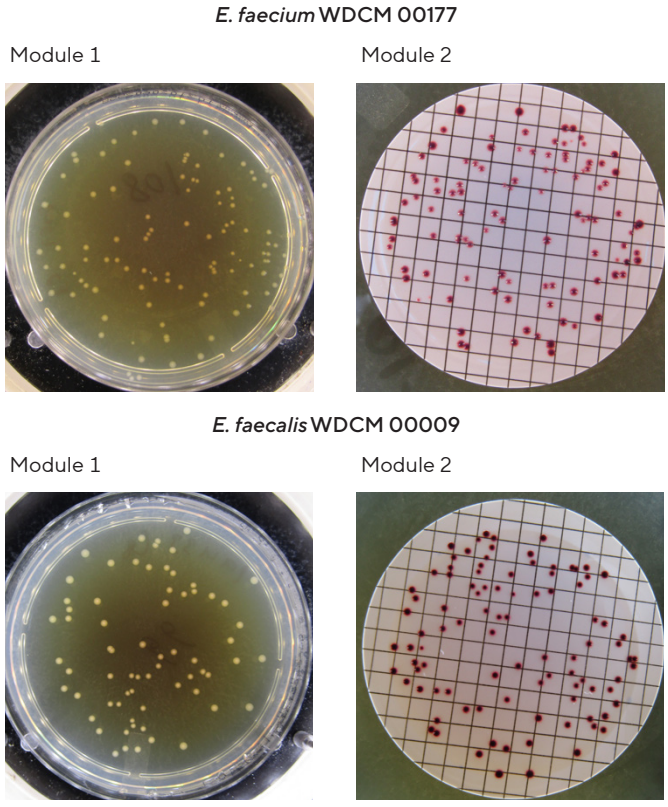

Figure 2: Exemplary results of *E. faecium* WDCM 00177 and *E. faecalis* WDCM 00009 grown on non-selective TSA (module 1) and on membrane filter on specific selective S&B agar (module 2).

The productivity ratio of *E. faecium* WDCM 00177 and *E. faecalis* WDCM 00009, when recovered on membrane filters in conjunction with S&B, was found to be well above the minimum required P_R of ≥ 0.5 (Figure 3). All tested combinations of membrane filters and the specific selective agar met the specification according to EN ISO 7704:2023.

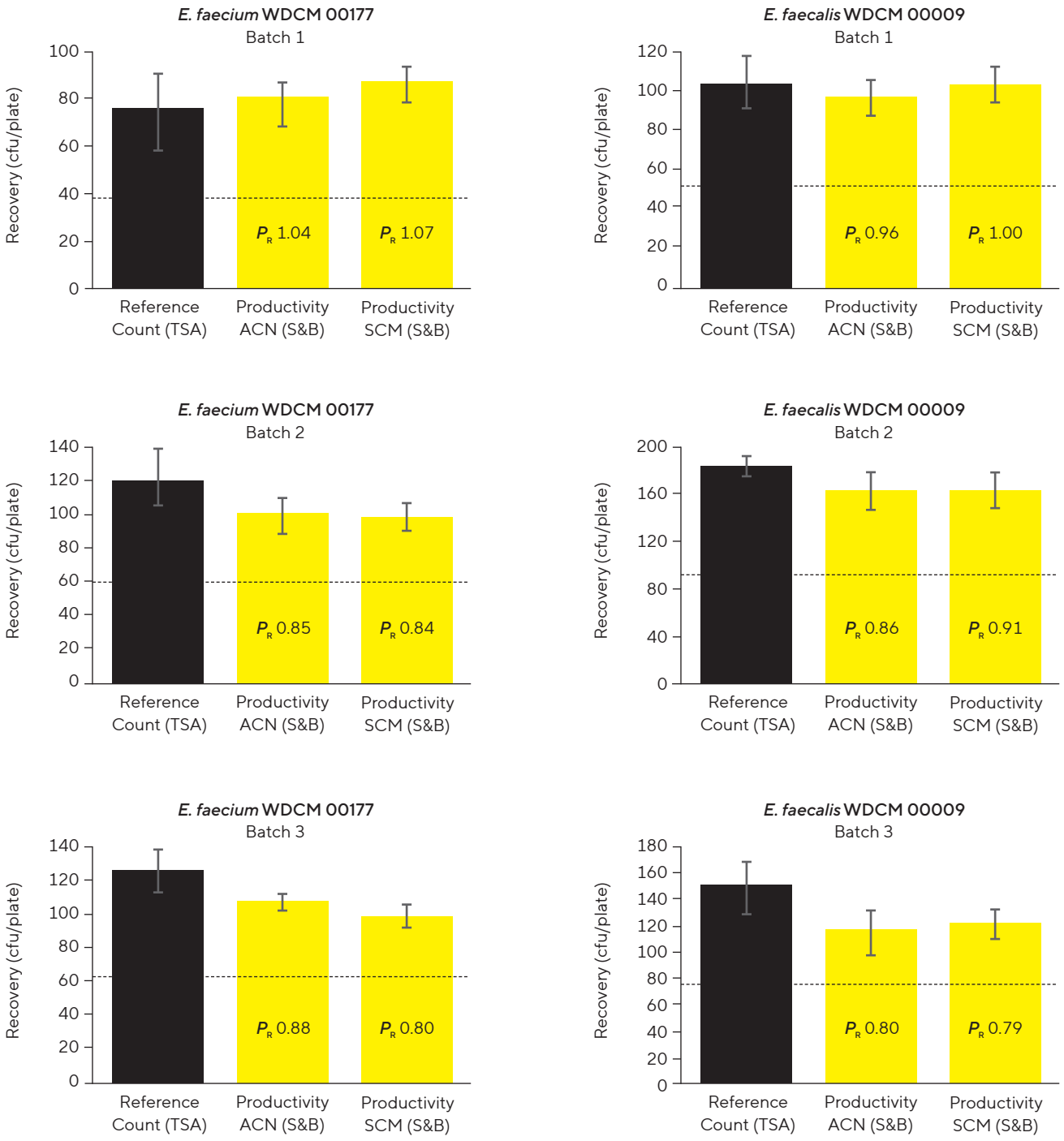

Figure 3: Performance testing for reference count determination on non-selective culture medium (TSA, as required by module 1) and productivity determination by using 114H6 membrane filters (ACN and SCM) with the specific selective culture medium (S&B, as required by module 2). The results for the productivity ratio (P_R) are depicted in the graph, with the dotted line indicating the minimum required P_R of ≥ 0.5.

Performance testing of batches according to module 2 includes the determination of productivity as well as selectivity and specificity, if applicable. Qualitative selectivity testing was conducted by membrane filtration of *S. aureus* WDCM 00034 and *E. coli* WDCM 00013 followed by incubation on S&B agar (Table 4). After the indicated period, no growth of the test strains on S&B was observed as expected.

Function	Incubation	Control strain	Method of control	Criteria	Results
Selectivity	44 ± 4 h 36 ± 2 °C	*S. aureus* WDCM 00034	Qualitative	Total inhibition	Conform
Selectivity	44 ± 4 h 36 ± 2 °C	*E. coli* WDCM 00013	Qualitative	Total inhibition	Conform

Table 4: Performance testing of S&B agar for qualitative selectivity according to module 2.

Conclusion

The performance testing conducted in this study demonstrated productivity ratios significantly above the minimum required P_R of 0.5 for each tested batch of membrane filters in conjunction with solid S&B media for *E. faecium* WDCM 00177 and *E. faecalis* WDCM 00009. Additionally, the qualitative selectivity testing for S&B agar according to module 2 was successfully passed by evaluating the relevant microorganisms. These results demonstrate EN ISO 7704:2023-compliant release of Sartorius cellulose nitrate high flow membrane filters, as exemplified by the specific standard EN ISO 7899-2.

For further information on EN ISO 7704:2023, please refer to our Best Practice Guide.[3]

This study is only a guidance and does not replace the requirement for internal method validation.

References

1. EN ISO 7704:2023 - Water quality — Requirements for the performance testing of membrane filters used for direct enumeration of microorganisms by culture methods

2. EN ISO 7899-2:2000 - Water quality — Detection and enumeration of intestinal enterococci — Part 2: Membrane filtration method

3. Best Practice Guide - New EN ISO 7704:2023 at a Glance

4. EN ISO 11133:2014 - Microbiology of food and animal feeding stuffs – Guidelines on preparation and production of culture media

5. EN ISO 8199:2018 - Water quality – General guidance on the enumeration of microorganisms by culture

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