

Keywords:

EN ISO 7704:2023, Microbiological Quality Control, Bioburden, Microbial Enumeration, Performance Testing, Membrane Filter, Water Quality, Media Performance, Water Testing

New EN ISO 7704:2023 at a Glance

Water quality – Requirements for the performance testing of membrane filters and media used for direct enumeration of microorganisms by culture methods

- Replaces the first edition ISO 7704:1985
- Applicable to membrane filters which are used for retention followed by direct enumeration of specific microorganisms on solid media or absorbent pads
- Applicable to the analysis of different types of water, including:
 - Drinking water, bottled water and other types of water with low numbers of microorganisms.
 - Water with expected higher numbers of microorganisms, e.g., surface or process water
- Meant to demonstrate suitability of each batch of the whole system, *i.e.*, the membrane filter together with the specific selective culture medium incl. the filtration step
- It applies to the following specific standards:
 - ISO 14189:** Enumeration of *Clostridium perfringens*
 - ISO 6461-2:** Detection and enumeration of the spores of sulfite-reducing anaerobes
 - ISO 7899-2:** Detection and enumeration of intestinal enterococci
 - ISO 9308-1:** Enumeration of *Escherichia coli* and coliform bacteria
 - ISO 11731:** Enumeration of *Legionella*
 - ISO 16266:** Detection and enumeration of *Pseudomonas aeruginosa*
- International Fruit and Vegetable Juice Association (IFU) refers to ISO 7704 e.g., for IFU MM No. 12: Method on the detection and enumeration of spore-forming thermo-acidophilic spoilage bacteria (*Alicyclobacillus* spp.)
- Applies to manufacturers & microbiological laboratories and describes the testing of membrane filters in combination with the specific selective culture medium
- Responsibility of the end user to ensure all required combinations of test strains, microbiological culture media, and membrane filters have been tested before use
- Testing to be performed for each batch of membrane filters with each batch of culture medium, no need of separate testing of membrane filters or culture media
- Performance testing (productivity, selectivity, specificity) aligned with ISO 11133
- For preparation and storage of culture media and diluents, follow procedures given in corresponding specific standards, ISO 11133 or ISO 8199.

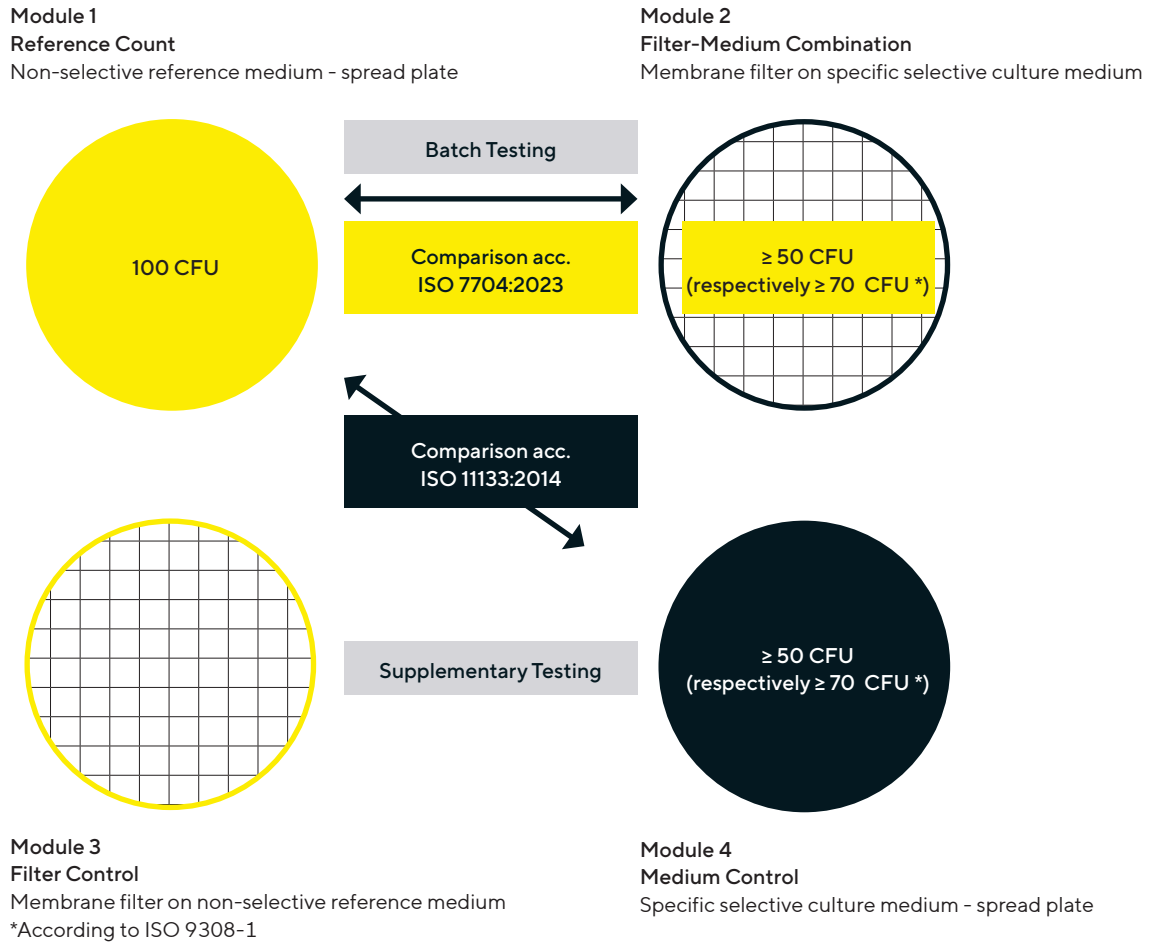


Figure 1: Batch testing (modules 1 and 2) and supplementary testing (modules 3 and 4) procedure.

Batch testing (modules 1 and 2) comprises the determination of the reference count, *i.e.*, total count of colonies on a non-selective reference medium obtained without usage of a membrane filter (module 1) and a simultaneous testing of units from a batch of membrane filters with units from a batch of specific selective culture medium for its intended use (module 2). From the counts achieved in modules 1 and 2, the productivity ratio can be calculated for the membrane filter used in combination with the specific selective culture medium (Table 1). Module 2 comprises also the test to evaluate the selectivity & specificity of the whole test system, the membrane filter in combination with the specific selective culture medium.

$P_R = \frac{N_s}{N_o}$	N_s is defined by the total count of colonies obtained from membrane filters in intended use with the specific culture medium according to module 2.
	N_o is defined by the total count of colonies obtained from plates of non-selective reference culture medium by direct inoculation without a membrane filter according to module 1.


Table 1: Calculation of the productivity ratio.

Batch testing needs to be adjusted to the procedure of the applicable standard in terms of culture media, incubation temperature and time as well as suitable control strain(s). If the criteria for the batch testing of membrane filters and specific selective culture medium given in the corresponding specific standard or ISO 11133 (*i.e.*, productivity: $P_R > 0.5$, respectively $P_R > 0.7$ according to ISO 9308-1) are not achieved, the laboratory should assess the discrepancies between the results by supplementary quantitative and/or qualitative testing.

Inoculum	Testing	Incubation	Counting & Results
<ul style="list-style-type: none"> Prepare suspension (inoculum) from working culture or (certified) reference material Suitable dilution to use as inoculum to be determined under standardized conditions For spread plate and membrane filtration technique, the inoculum volume should be the same and in the range of 0.1 - 0.5 mL 	<ul style="list-style-type: none"> Reference count on a non-selective reference culture medium by spread plating technique (module 1) Productivity, selectivity and specificity using membrane filters with the specific selective culture medium by membrane filtration technique (module 2) 	<ul style="list-style-type: none"> Prepare plates (90 mm diameter), each containing 18 ± 2 mL of culture medium Dry surface of medium before use Place membrane filters on culture medium ensuring no air is trapped underneath Allow spread plates to absorb inoculum Invert plates for incubation 	<ul style="list-style-type: none"> Quantitative testing: productivity ratio is determined comparing reference count with count from membrane filter on the specific culture medium Selectivity testing: assess growth of non-target control strains of membrane filters with specific culture medium Specificity testing: assess e.g., physiological characteristics to differentiate microorganisms

Target and non-target control strains to be used for productivity, selectivity and specificity testing, non-selective reference and specific selective culture media as well as incubation conditions and specifications are stated in corresponding standards

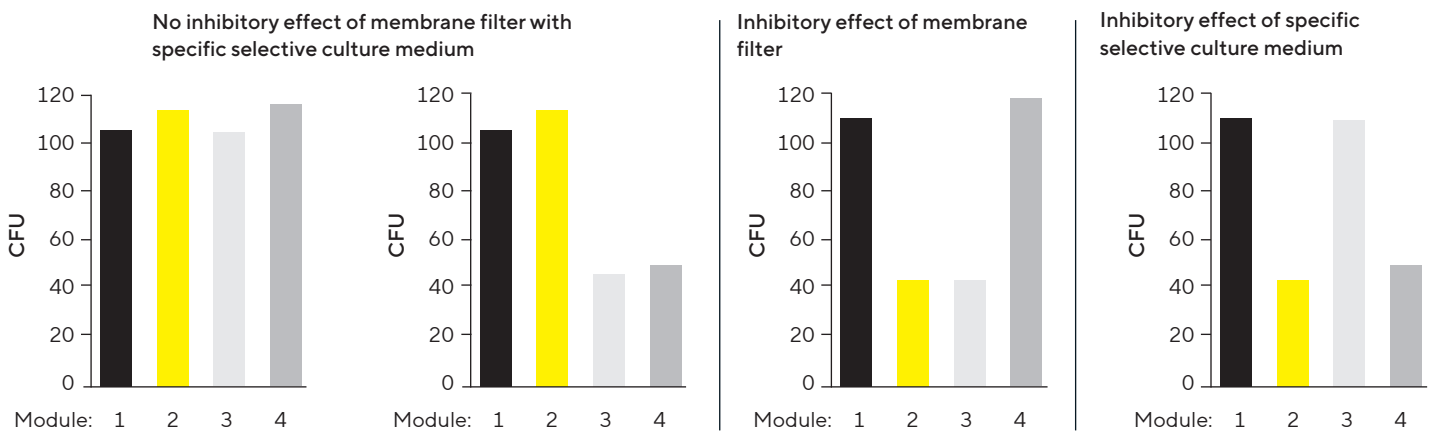


 Release of the batch of membrane filter and batch of the specific culture medium for combined use

Supplementary quantitative testing (modules 1-4) is used when a new type of membrane filter or a new manufacturer is tested initially, or when a problem in the day-to-day use or batch testing of membrane filters is noticed.

Preparation of suspension (inoculum) for the test by using a working culture or (certified) reference material and simultaneous testing of modules 1 - 4

Module 1	Module 2	Module 3	Module 4
<p>Determining the reference count</p> <p>Using spread plate technique on a non-selective culture medium.</p>	<p>Productivity, selectivity and specificity of the membrane filter and specific selective culture medium combination</p> <p>Using membrane filtration technique with the membrane filter used with the specific selective culture medium.</p>	<p>Detecting target organism inhibition due to the membrane filter</p> <p>Using membrane filtration technique with the membrane filter used with a non-selective culture medium.</p>	<p>Detecting target organism inhibition due to the specific culture medium</p> <p>Using spread plate technique on the specific selective culture medium.</p>



As long as productivity (P_R), selectivity and specificity tests are within specification, a low productivity in supplementary testing does not disqualify the combination of membrane filter and culture medium. Batch testing (module 1 & 2) can meet specifications, although P_R of the testing of module 3 & 4 are < 0.7 respectively < 0.5 .




* Module 1 is not needed when using inoculum prepared by certified reference material or for the qualitative testing of selectivity and specificity



Supplementary qualitative testing of membrane filters and media is appropriate to consider when discrepancies in colony appearance occur between different membrane filter and/or culture medium brands, types or lots. Tests should include properties such as hydrophobicity of membrane filters, grid line growth inhibition and grid line colony proliferation. Additionally, factors such as colony color, irregularity, convexity, size and other relevant characteristics for culture medium should be evaluated.

Sartorius EN ISO 7704:2023 compliance

Sartorius' filtration products support your EN ISO 7704:2023 compliant water testing. EN ISO 7704:2023-certified nitrocellulose membrane filters guarantee highest quality and performance in combination with culture media. Sterile single-use or reusable filtration devices support convenient microbial enumeration testing.

Product Information

Product Type	Description	Pack Size	Order No.
CN Membrane Filter			
	Sterile, single packaged 0.45 µm High Flow white & gridded Ø47mm	100	114H6--47----ACN
	Sterile, single packaged 0.45 µm High Flow white & gridded Ø47mm	1000	114H6--47----ACR
	Microsart® e.motion Membrane Filters, single sterile Dispenser packaging, 0.45 µm High Flow white & gridded Ø47mm	3 x 100	114H6Z-47----SCM
	Microsart® e.motion Membrane Filters, single sterile Dispenser packaging, 0.45 µm High Flow white & gridded Ø47mm	4 x 250	114H6Z-47----SJR
	Sterile, single packaged 0.45 µm High Flow black & gridded Ø47mm	100	130H6--47----ACN
	Sterile, single packaged 0.45 µm High Flow black & gridded Ø47mm	1000	130H6--47----ACR
	Microsart® e.motion Membrane Filters, single sterile Dispenser packaging, 0.45 µm High Flow black & gridded Ø47mm	3 x 100	130H6Z-47----SCM
	Microsart® e.motion Membrane Filters, single sterile Dispenser packaging, 0.45 µm High Flow black & gridded Ø47mm	4 x 250	130H6Z-47----SJR
	Sterile, single packaged 0.45 µm white & gridded Ø47mm	100	11406--47----ACN
	Sterile, single packaged 0.45 µm white & gridded Ø47mm	1000	11406--47----ACR
	Microsart® e.motion Membrane Filters, single sterile Dispenser packaging, 0.45 µm white & gridded Ø47mm	3 x 100	11406Z-47----SCM
	Sterile, single packaged 0.45 µm black & gridded Ø47mm	100	13006--47----ACN
	Sterile, single packaged 0.45 µm black & gridded Ø47mm	1000	13006--47----ACR
	Microsart® e.motion Membrane Filters, single sterile Dispenser packaging, 0.45 µm black & gridded Ø47mm	3 x 100	13006Z-47----SCM
Reusable Filtration Funnel			
	Biosart® 250 Funnels, sterile, reusable 250 mL funnel, individually, packaged	50	16407--25----ACK
	Biosart® 250 Funnels, sterile, reusable 250 mL funnel, bulk packaging	50	16407--25----ALK
Single use Filtration Funnel			
	Microsart® Funnels 100, sterile packaged in bags, 100 mL, ready-to-use	100	16A07--10-----N
	Microsart® Funnels 250, sterile packaged in bags, 250 mL, ready-to-use	100	16A07--25-----N


Product Type	Description	Pack Size	Order No.
Sterile Monitors with Membrane and Cellulose Pad under the Membrane			
	Biosart® 100 Monitors, 100 mL, 47 mm diameter, 0.45 µm High Flow, white & gridded	4 x 12 tray	1640147-H6-V--K
	Biosart® 100 Monitors, 100 mL, 47 mm diameter, 0.45 µm white & gridded	4 x 12 tray	16401--47-06-V--K
	Biosart® 100 Monitors, 100 mL, 47 mm diameter, 0.45 µm black & gridded	4 x 12 tray	1640347-06-V--K
	Biosart® 100 Monitors, 100 mL, 47 mm diameter, 0.45 µm white & gridded	48 single sterile	16401-47-06--ACK sterile
	Biosart® 100 Monitors, 100 mL, 47 mm diameter, 0.45 µ black & gridded	48 single sterile	16403-47-06--ACK sterile
	Biosart® 100 Monitors, 100 mL, 47 mm diameter, 0.45 µm High Flow, white & gridded	48	16401-47-H6----K
	Biosart® 100 Monitors, 100 mL, 47 mm diameter, 0.45 µm, white & gridded	48	16401-47-06----K
Biosart® 100 Monitors, 100 mL, 47 mm diameter, 0.45 µm, black & gridded	48	16403-47-06----K	
Sterile Filtration Funnel with Membrane			
	Microsart® @Filter 100 – Bag version, 100 mL Capacity, sterile packed in bags; CN white & gridded 0.45µm High Flow	60	16D00--10-H6--BL
	Microsart® @Filter 100 – Bag version, 100 mL Capacity, sterile packed in bags; CN white & gridded 0.45µm High Flow	240	16D01--10-H6--BJ
	Microsart® @Filter 100 – Bag version, 100 mL Capacity, sterile packed in bags; CN black & gridded 0.45µm High Flow	60	16D03--10-H6--BL
	Microsart® @Filter 100, Tray version, 100 mL Capacity, sterile packed in bags, with covers; CN white & gridded	24	16D01--10-H6--TG
	Microsart® @Filter 100, Tray version, 100 mL Capacity, sterile packed in bags, with covers; CN black & gridded	24	16D03--10-H6--TG

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