

# Study of the use of a water purification system for the preparation and performance testing of microbiological culture media according to EN ISO 11133

## Authors

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## Keywords

microbiology, quality control, testing, culture media, deionized water, water purification, standard, EN ISO 11133, Milli-Q IX

## ABSTRACT

Microbiology testing is of major importance to guarantee the safety and quality of food. Laboratories performing these tests are required to deliver accurate and reliable results and meet regulatory requirements while under time pressure. Many tests and procedures depend upon culture media being capable of providing consistent and reproducible results. Amongst those, the EN ISO 11133 standard establishes the context that ensures the quality of culture media and specifies the requirements for media preparation when used for the microbiological analysis of food, animal feed and water.<sup>1</sup> In order to be compliant with the standard, each element of the process must meet specific requirements. Therefore, the quality of the water used is important, as it constitutes the predominant component in any media formulation.

The goal of this study was to investigate whether a modern water purification system installed in a microbiology laboratory could advantageously replace centrally produced deionized water when preparing and testing microbiological media. Seven different dehydrated culture media compliant with the corresponding EN ISO/FDA Bacteriological Analytical Manual (BAM)/USDA-FSIS/APHA and other standards and methods were involved in performance testing. The study was performed by a Quality Control (QC) laboratory for microbiological products, which is accredited by the German accreditation authority



DAkKS as a registered test laboratory according to DIN EN ISO/IEC 17025<sup>2</sup> for the performance testing of media for microbiology according to DIN EN ISO 11133.

The results revealed that culture media prepared with water from the Milli-Q® IX system performed similarly as the culture media prepared with the laboratory's routinely used deionized water. This water purification system enabled the laboratory to prepare media performing as required by the standard, while bringing additional benefits such as ease of use, convenience, data traceability and autonomy.

## INTRODUCTION

Culture media are used in all traditional microbiological culture techniques and for many alternative techniques. It is essential to use culture media of proven quality for reliable microbiological analysis. For this reason, laboratories using culture media need to confirm the acceptability of each batch of medium and must ensure that the medium is “fit-for-purpose” and can produce consistent results. These three criteria are a crucial part of internal quality control procedures. With appropriate documentation, they will allow effective monitoring of culture media and will contribute to the production of both accurate and reliable data.

The EN ISO 11133:2014 standard,<sup>1</sup> including its amendments (Amd 1:2018 and Amd 2:2020) is among the required standards for reliable microbiological analysis. Entitled “*Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media*”, it defines terms related to quality assurance of culture media and specifies the requirements for the preparation of culture media intended for the microbiological analysis of food, animal feed, and samples from the food or feed production environment, as well as all kinds of water intended for consumption or used in food production. It sets criteria and describes methods for the performance testing of culture media. These requirements are applicable to all categories of culture media prepared for use in laboratories performing microbiological analyses. EN ISO 11133<sup>1</sup> is applicable to end-users of ready-to-use media, to laboratories that prepare media for their own use, and to manufacturers of culture media.

As a full European and International (EN ISO) standard, EN ISO 11133<sup>1</sup> is now mandatory for all laboratories accredited on methods of (EN) ISO standards for microbiological testing of food, animal feed or water. Nearly all specific (EN) ISO standards related to food and water microbiology now include a normative reference to EN ISO 11133, making EN ISO 11133 indispensable for their application. For instance, ISO 17025<sup>2</sup> accreditation specifies the quality management and technical requirements that laboratories must meet to demonstrate technical competency and compliance with regulatory standards. ISO 17025-accredited test laboratories registered for these methods must use EN ISO 11133<sup>1</sup> for the quality assurance of the



culture media used in the analyses. The general EN ISO 7218<sup>3</sup> (food microbiology) and EN ISO 8199<sup>4</sup> (water microbiology) standards also give a normative reference to EN ISO 11133. Similarly, manufacturers or contract testing laboratories must follow EN ISO 11133 strictly if holding an ISO 17025 accreditation on the EN ISO 11133 methods for the performance testing of culture media in microbiology.

Water can potentially affect culture media performance since it is the largest component of microbiological media by volume. The present study was therefore developed to identify the most suitable water purification solution for food microbiology testing laboratories in terms of water quality, ease of use, autonomy and convenience. The study was performed by the MilliporeSigma Life Science QC laboratory for microbiological products in Darmstadt, Germany. The laboratory is accredited by the German accreditation authority DAkkS as registered test laboratory D-PL-15185-01-00 according to DIN EN ISO/IEC 17025<sup>2</sup> for the performance testing of media for microbiology according to DIN EN ISO 11133.

## Goal of the study

This study was designed to compare the performance of several types of microbiological culture media prepared with either the routinely-used deionized (DI) water of the QC laboratory obtained from a distribution loop, or water freshly purified by a Milli-Q® IX water purification system installed in the laboratory. Seven different dehydrated culture media were selected (**Table 1**) to obtain representative results. All are

compliant with the corresponding EN ISO and/or other standards. Each culture medium was prepared according to manufacturer's instructions and inoculated with specific microorganisms as required by the specific standards and/or EN ISO 11133.<sup>1</sup> Performance of each medium was assessed according to the EN ISO 11133 standard and/or by the specific standard.

**Table 1:** Application of culture media used in this study and the standards specifying them.

Culture medium	Culture media description	Standard specifying the medium
<b>Listeria agar according to Ottaviani and Agosti</b>	For the isolation and differentiation of <i>Listeria monocytogenes</i> and other <i>Listeria</i> spp. from food and animal feed, environmental samples in the area of food production and food handling, and other materials.	ISO 11290, FDA-BAM, APHA
<b>Violet red bile lactose (VRBL) agar</b>	For the detection and colony counting of coliform bacteria from food and animal feed, water and other materials.	ISO 4832, FDA-BAM, APHA
<b>Malt extract agar</b>	For the detection, isolation and enumeration of yeasts and molds in food products and other materials, and for their maintenance.	-
<b>de Man, Rogosa and Sharpe (MRS) agar</b>	For the isolation, enumeration and cultivation of <i>Lactobacillus</i> spp. and other mesophilic lactic acid bacteria from all types of materials.	ISO 15214, APHA
<b>Rappaport-Vassiliadis medium with soya (RVS) broth</b>	For the selective enrichment of <i>Salmonella</i> spp. from food and animal feed, water and other materials.	ISO 6579-1, ISO 19250
<b>Brilliant green bile lactose bile (BRILA) broth</b>	For the selective enrichment, enumeration and confirmation of <i>Escherichia coli</i> and other fecal coliform organisms from food and animal feed, water and other materials.	ISO 4831, ISO 4832, FDA-BAM, APHA
<b>Modified tryptone soya broth (mTSB) with novobiocin</b>	For the selective enrichment of <i>Escherichia coli</i> serogroup O157 in food and feed samples.	ISO 16654

## EXPERIMENTAL METHODS

### Water used to prepare media

Each medium was prepared in duplicate by dissolving dehydrated medium in water from two different sources: DI water readily available in the QC laboratory and water from a Milli-Q® IX 7003 water purification system.

The DI water available in the laboratory was centrally produced by a combination of reverse osmosis, ion exchange and a bactericidal UV lamp and delivered to the laboratory by a distribution loop. The water conductivity remained below 2 µS/cm throughout the study and the microbial level was < 10<sup>2</sup> colony forming units (cfu)/mL, as required by the EN ISO 11133 standard<sup>1</sup> (see **Appendix**). In the present study, “DI water” refers to the readily available water obtained from the central production and routinely used in the laboratory.

The second source of water was a Milli-Q® IX 7003 water purification system that was installed in the laboratory. This system was directly connected to tap water and combined several water purification technologies to deliver purified water: reverse osmosis, Elix® electrodeionization and bactericidal UVC LED lamps. Freshly purified water was collected from the system’s E-POD® dispenser, which was fitted with a Millipak® 0.22 µm filter to ensure low microbial levels. This system is validated to deliver purified water with a conductivity < 0.2 µS/cm, typically 0.1 µS/cm at 25°C (equivalent to a resistivity > 5 MOhm.cm, typically 10-15 MOhm.cm), and microbial level < 10<sup>2</sup> cfu/mL, and fits the requirements of the ISO 11133 standard<sup>1</sup> (see **Appendix**). In the following paragraphs, “Milli-Q® IX water” refers to the water produced by the Milli-Q® IX 7003 system.

### Culture media preparation

The seven dehydrated culture media used in this study are described in **Table 1**. The standards specifying the media and the ones specifying their relative performance testing are reported in **Table 2**, as well as the control stains, and the WDCM and ATCC® numbers.

The media were prepared following the instructions of the manufacturer. Specifically, the required mass of the dehydrated media was added to 500 mL of DI water or Milli-Q® IX water. All media were dissolved, heated and, if required, autoclaved as needed according to manufacturer’s instructions.

### Culture media evaluation: physical and chemical quality control

Each prepared culture medium was assessed by visual inspection to ensure that it conformed to stated recommendations, e.g. appearance, color, homogeneity, gel consistency and pH.

### Microbiological performance testing

Growth and inhibition were assessed by either quantitative or qualitative methods as described in the EN ISO 11133 standard.<sup>1</sup> Productivity is the level of recovery of a target microorganism from the culture medium under defined conditions. Selectivity is the degree of inhibition of a non-target microorganism on or in a selective culture medium under defined conditions. Specificity is the demonstration, under defined conditions, that non-target organisms, if able to grow on the medium, do not show the same visual characteristics as target microorganisms.

#### 1. Bacteria, yeasts and mold preparation and inoculation

Control strains were obtained from ATCC® (American Type Culture Collection) and were selected according to the requirements of the EN ISO 11133 standard<sup>1</sup> and/or other specific standards. They were diluted to the desired number of organisms for inoculation for testing on productivity, specificity and selectivity. Performance evaluation and interpretation of the results were obtained following the specifications given by EN ISO 11133.<sup>1</sup>

#### 2. Performance testing

##### a. Quantitative methods

Quantitative productivity for solid culture media was measured by quantitative methods as described in EN ISO 11133.<sup>1</sup>

##### b. Qualitative methods

Productivity, specificity and selectivity were determined qualitatively using the methods as described by EN ISO 11133.<sup>1</sup>

**Table 2:** List of control strains for performance testing of culture media in the present study in accordance with the List of control strains for performance testing of culture media and reagents from published standards from ISO/TC 34/SC 9 & SC 5 (food microbiology) and ISO/TC 147/SC 4 (water microbiology).

Culture medium	Standard specifying the medium	Standard specifying the performance testing	Control strains specified by the standard	WDCM numbers specified by the standard	WDCM numbers used in this study
<b>Listeria agar according to Ottaviani and Agosti</b>	ISO 11290-1:2017 ISO 11290-2:2017	ISO 11290-1:2017 ISO 11290-2:2017	<i>Listeria monocytogenes</i> serovar 4b	00021 <sup>b</sup>	00021
			<i>Listeria monocytogenes</i> serovar 1/2a	00109 <sup>a</sup>	00109
			<i>Escherichia coli</i>	00012 <sup>d</sup> 00013 <sup>d</sup>	00012 00013
			<i>Enterococcus faecalis</i>	00009 <sup>d</sup> 00087 <sup>d</sup>	00009 00087
			<i>Listeria innocua</i>	00017 <sup>b</sup>	00017
<b>Violet red bile lactose (VRBL) agar</b>	ISO 4832:2006	EN ISO 11133:2014	<i>Escherichia coli</i>	00012 <sup>b</sup> 00013 <sup>a</sup>	00012 00013
			<i>Enterococcus faecalis</i>	00009 <sup>d</sup> 00087 <sup>a</sup>	00009 00087
			<i>Pseudomonas aeruginosa</i>	00025 <sup>b</sup>	00025
<b>Malt extract agar</b>	-	-	-	-	-
<b>de Man, Rogosa and Sharpe (MRS) agar</b>	ISO 15214:1998	EN ISO 11133:2014	<i>Lactobacillus sakei</i>	00015 <sup>b</sup>	00015
			<i>Lactococcus lactis</i>	00016 <sup>b</sup>	00016
			<i>Pediococcus pentosaceus</i>	00158 <sup>a</sup>	00158
			<i>Escherichia coli</i>	00012 <sup>d</sup> 00013 <sup>d</sup>	00012 00013
			<i>Bacillus cereus</i>	00001 <sup>a</sup>	00001
<b>Rappaport-Vassiliadis medium with soya (RVS) broth</b>	ISO 6579-1:2017	ISO 6579-1:2017	<i>Salmonella</i> Typhimurium /Enteritidis	00030 <sup>c,d</sup> 00031 <sup>c,d</sup>	00030 00031
			<i>Escherichia coli</i>	00012 <sup>d</sup> 00013 <sup>d</sup>	00012 00013
			<i>Pseudomonas aeruginosa</i>	00025 <sup>b</sup>	00025
			<i>Enterococcus faecalis</i>	00009 <sup>d</sup> 00087 <sup>d</sup>	00009 00087
<b>Brilliant green bile lactose bile (BRILA) broth</b>	ISO 4831:2006 ISO 4832:2006	EN ISO 11133:2014 / Amd 2:2020	<i>Escherichia coli</i>	00012 <sup>d</sup> 00013 <sup>d</sup> 00090 <sup>d</sup> 00179 <sup>d</sup>	00012 00013 00090 00179
			<i>Enterococcus faecalis</i>	00009 <sup>d</sup> 00087 <sup>d</sup> 00176 <sup>d</sup>	00009 00087 00176
			-	-	-
<b>Modified tryptone soya broth (mTSB) with novobiocin</b>	ISO 16654:2001	-	-	-	-

<sup>b</sup>Strain to be used as minimum; <sup>c</sup>Some national restrictions and directions may require the use of a different serovar. Refer to national requirements relating to the choice of *Salmonella* serovars; <sup>d</sup>Strain free of choice; one of the positive strains has to be used as a minimum; <sup>a</sup>Strain optional. (Only the required footnotes are reported here).



## RESULTS AND DISCUSSION

The purpose of the present study was to prepare culture media and to test their performance according to EN ISO 11133<sup>1</sup> using water from two alternative sources. Solid and liquid culture media for important pathogens and hygiene indicators were included in this study, such as *Listeria*, *Salmonella*, *Escherichia coli* serogroup O157 and coliforms, as well as culture media for yeast, molds and lactic acid bacteria.

### Physical and chemical evaluation of the prepared culture media

The seven culture media studied were prepared with water from the two alternative sources. **Table 3** describes the physical parameters and the expected specifications, as well as the results obtained in both conditions. In each case, the media prepared with water from the Milli-Q® IX system matched the required specifications and gave similar outcomes as media prepared with the centrally produced DI water of the QC laboratory.

**Table 3:** Physical parameters and specifications of the seven culture media used in the study, as well as results obtained with either DI water or water from a Milli-Q® IX system.

Media	Physical parameters	Specifications	DI water results	Milli-Q® IX water results
<b>Listeria agar according to Ottaviani and Agosti</b>	Appearance (clarity)	slightly opalescent to opalescent	opalescent	opalescent
	Appearance (color)	yellowish	yellowish	yellowish
	pH value (at 25°C)	7.0 – 7.4	7.1	7.1
<b>Violet red bile lactose (VRBL) agar</b>	Appearance (clarity)	clear	clear	clear
	Appearance (color)	red	red	red
	pH value (at 25°C)	7.2 – 7.6	7.4	7.4
	Solidification behavior (2 h at 45°C)	liquid	liquid	liquid
<b>Malt extract agar</b>	Appearance (clarity)	clear to slightly opalescent	almost clear	almost clear
	Appearance (color)	brown	brown	brown
	pH value (at 25°C)	5.4 – 5.8	5.5	5.5
<b>de Man, Rogosa and Sharpe (MRS) agar</b>	Appearance (clarity)	clear	clear	clear
	Appearance (color)	brown	brown	brown
	pH value (at 25°C)	5.6 – 5.8	5.6	5.6
	Solidification behavior (2 h at 45°C)	liquid	liquid	liquid
<b>Rappaport-Vassiliadis medium with soya (RVS) broth</b>	Appearance (clarity)	clear	clear	clear
	Appearance (color)	dark blue	dark blue	dark blue
	pH value (at 25°C)	5.0 – 5.4	5.4	5.4
<b>Brilliant green bile lactose bile (BRILA) broth</b>	Appearance (clarity)	clear	clear	clear
	Appearance (color)	green	green	green
	pH value (at 25°C)	7.0 – 7.4	7.2	7.2
<b>Modified tryptone soya broth (mTSB) with novobiocin</b>	Appearance (clarity)	clear	clear	clear
	Appearance (color)	yellowish brown	yellowish brown	yellowish brown
	pH value (at 25°C)	7.2 – 7.6	7.3	7.2

### Outcomes of the performance testing of the prepared media

All the culture media prepared and tested met the required criteria for productivity, selectivity and specificity, when applicable. Quantitative productivity tests met the required criteria: productivity ratio (PR) ≥ 50% for selective media and PR ≥ 70% for non-selective media. **Table 4** and **Table 5** report the results obtained in the study. Qualitative and quantitative tests of the culture media led to results in accordance with the standard requirements with DI water used in the QC laboratory as well as with water purified by the Milli-Q® IX system.

**Table 4:** Results of the performance tests of agar-based media prepared with either DI water or water from a Milli-Q® IX system.

Media	Function / Method of control	Strains tested	Specifications	DI water results	Milli-Q® IX water results
<b>Listeria agar according to Ottaviani and Agosti</b>	Productivity / Quantitative	<i>Listeria monocytogenes</i> ATCC® 13932™ [WDCM 00021] <i>Listeria monocytogenes</i> ATCC® 35152™ [WDCM 00109]	Recovery rate ≥ 50%	<i>L. monocytogenes</i> ATCC® 13932™: 117%, Blue green colonies with opaque halo <i>L. monocytogenes</i> ATCC® 35152™: 74%, Blue green colonies with opaque halo <b>PASS</b>	<i>L. monocytogenes</i> ATCC® 13932™: 124%, Blue green colonies with opaque halo <i>L. monocytogenes</i> ATCC® 35152™: 83%, Blue green colonies with opaque halo <b>PASS</b>
	Selectivity / Qualitative	<i>Escherichia coli</i> ATCC® 8739™ [WDCM 00012] <i>Escherichia coli</i> ATCC® 25952™ [WDCM 00013] <i>Enterococcus faecalis</i> ATCC® 29212™ [WDCM 00087] <i>Enterococcus faecalis</i> ATCC® 19433™ [WDCM 00009]	Total inhibition	Total inhibition <b>PASS</b>	Total inhibition <b>PASS</b>
	Specificity / Qualitative	<i>Listeria innocua</i> ATCC® 33090™ [WDCM 00017]	No limit	Growth, blue-green colonies without opaque halo <b>PASS</b>	Growth, blue-green colonies without opaque halo <b>PASS</b>
<b>Violet red bile lactose (VRBL) agar</b>	Productivity / Quantitative	<i>Escherichia coli</i> ATCC® 8739™ [WDCM 00012] <i>Escherichia coli</i> ATCC® 25922™ [WDCM 00013] <i>Enterobacter cloacae</i> ATCC® 13047™ [WDCM 00083]	Recovery rate ≥ 50%	<i>E. coli</i> ATCC® 8739™: 82% <i>E. coli</i> ATCC® 25922™: 105% <i>E. cloacae</i> : 85% All: purplish-red colonies with or without precipitation halo <b>PASS</b>	<i>E. coli</i> ATCC® 8739™: 103% <i>E. coli</i> ATCC® 25922™: 102% <i>E. cloacae</i> : 93% All: purplish-red colonies with or without precipitation halo <b>PASS</b>
	Selectivity / Qualitative	<i>Enterococcus faecalis</i> ATCC® 29212™ [WDCM 00087] <i>Enterococcus faecalis</i> ATCC® 19433™ [WDCM 00009]	Total inhibition	Total inhibition <b>PASS</b>	Total inhibition <b>PASS</b>
	Specificity / Qualitative	<i>Pseudomonas aeruginosa</i> ATCC® 27853™ [WDCM 00025]	Growth, no limit; colorless to beige colonies	Good growth, colorless to beige colonies <b>PASS</b>	Good growth, colorless to beige colonies <b>PASS</b>
<b>Malt extract agar</b>	Productivity / Quantitative	<i>Candida albicans</i> ATCC® 10231™ [WDCM 00054] <i>Saccharomyces cerevisiae</i> ATCC® 9763™ [WDCM 00058] <i>Saccharomyces cerevisiae</i> ATCC® 9080™ <i>Rhodotoula mucilaginosa</i> DSM 70403™	Recovery rate ≥ 70%	<i>C. albicans</i> : 82% <i>S. cerevisiae</i> ATCC® 9763™: 107% <i>S. cerevisiae</i> ATCC® 9080™: 87% <i>R. mucilaginosa</i> : 113% <b>PASS</b>	<i>C. albicans</i> : 82%, <i>S. cerevisiae</i> ATCC® 9763™: 100%, <i>S. cerevisiae</i> ATCC® 9080™: 102%, <i>R. mucilaginosa</i> : 116% <b>PASS</b>
	Specificity / Qualitative	<i>Geotrichum candidum</i> DSM 1240™ <i>Penicillin commune</i> ATCC® 10428™ <i>Aspergillus brasiliensis</i> ATCC® 16404™ [WDCM 00053]	Good to very good growth	Very good growth <b>PASS</b>	Very good growth <b>PASS</b>
		<i>Trichophyton ajelloi</i> ATCC® 28454™	Moderate to good growth	Good growth <b>PASS</b>	Good growth <b>PASS</b>
<b>de Man, Rogosa and Sharpe (MRS) agar</b>	Productivity / Quantitative	<i>Lactobacillus acidophilus</i> ATCC® 4356™ [WDCM 00098]* <i>Lactobacillus sakei</i> ATCC® 15521™ [WDCM 00015] <i>Lactococcus lactis</i> ATCC® 19435™ [WDCM 00016] <i>Pediococcus pentosaceus</i> ATCC® 33316™ [WDCM 00158] <i>Pediococcus damnosus</i> ATCC® 29358™ [WDCM 00022]*	Recovery rate ≥ 70%	<i>L. acidophilus</i> : 96% <i>L. sakei</i> : 106% <i>L. lactis</i> : 97% <i>P. pentosaceus</i> : 91% <i>P. damnosus</i> : 102% <b>PASS</b>	<i>L. acidophilus</i> : 106% <i>L. sakei</i> : 100% <i>L. lactis</i> : 99%, <i>P. pentosaceus</i> : 88% <i>P. damnosus</i> : 108% <b>PASS</b>
	Selectivity / Qualitative	<i>Escherichia coli</i> ATCC® 25922™ [WDCM 00013] <i>Escherichia coli</i> ATCC® 8739™ [WDCM 00012] <i>Bacillus cereus</i> ATCC® 11778™ [WDCM 00001]	Total inhibition	Total inhibition <b>PASS</b>	Total inhibition <b>PASS</b>
		<i>Bifidobacterium bifidum</i> ATCC® 11863™	Good growth	Good growth <b>PASS</b>	Good growth <b>PASS</b>

\*Additional strains used in the experiments of the study. Strains tested for malt extract agar were chosen by the QC laboratory.

**Table 5:** Results of the performance tests of broth prepared with either DI water or water from a Milli-Q® IX system.

Media	Function / Method of control	Strains tested	Specifications	DI water results	Milli-Q® IX water results
<b>Rappaport-Vassiliadis medium with soya (RVS) broth</b>	Productivity / Qualitative	<i>Salmonella Typhimurium</i> ATCC® 14028™ [WDCM 00031] + <i>Escherichia coli</i> ATCC® 25922™ [WDCM 00013] + <i>Pseudomonas aeruginosa</i> ATCC® 27853™ [WDCM 00025]	> 10 colonies with black center on XLD agar	> 10 colonies with black center on XLD agar <b>PASS</b>	> 10 colonies with black center on XLD agar <b>PASS</b>
	Productivity / Qualitative	<i>Salmonella Enteritidis</i> ATCC® 13076™ [WDCM 00030] + <i>Escherichia coli</i> ATCC® 8739™ [WDCM 00012] + <i>Pseudomonas aeruginosa</i> ATCC® 27853™ [WDCM 00025]	> 10 colonies with black center on XLD agar	> 10 colonies with black center on XLD agar <b>PASS</b>	> 10 colonies with black center on XLD agar <b>PASS</b>
	Selectivity / Qualitative	<i>Escherichia coli</i> ATCC® 8739™ [WDCM 00012] <i>Escherichia coli</i> ATCC® 25922™ [WDCM 00013]	Partial inhibition ≤ 100 colonies on Tryptic Soy Agar (TSA)	For both, partial inhibition: ≤ 100 colonies on TSA; <b>PASS</b>	For both, partial inhibition: ≤ 100 colonies on TSA; <b>PASS</b>
<b>Brilliant green bile lactose bile (BRILA) broth</b>	Productivity / Qualitative	<i>Escherichia coli</i> ATCC® 25922™ [WDCM 00013] <i>Escherichia coli</i> ATCC® 8739™ [WDCM 00012] <i>Citrobacter freundii</i> ATCC® 43864™ [WDCM 00006]*	Turbidity and gas	For all 3: turbidity and gas <b>PASS</b>	For all 3: turbidity and gas <b>PASS</b>
	Selectivity / Qualitative	<i>Enterococcus faecalis</i> ATCC® 29212™ [WDCM 00087] <i>Enterococcus faecalis</i> ATCC® 19433™ [WDCM 00009]	Partial inhibition, no gas production	For both, total inhibition, no gas production <b>PASS</b>	For both, total inhibition, no gas production <b>PASS</b>
	Selectivity / Qualitative	<i>Staphylococcus aureus</i> ATCC® 6538™ [WDCM 00032]* <i>Bacillus cereus</i> ATCC® 11778™ [WDCM 00001]*	Total inhibition, no gas production	For both, total inhibition, no gas production <b>PASS</b>	For both, total inhibition, no gas production <b>PASS</b>
<b>Modified tryptone soya broth (mTSB) with novobiocin</b>	Productivity / Qualitative	<i>Escherichia coli</i> ATCC® 35150™ + <i>Staphylococcus aureus</i> ATCC® 25923™ [WDCM 00034] <i>Escherichia coli</i> ATCC® 700728™ [WDCM 00014] + <i>Staphylococcus aureus</i> ATCC® 25923™ [WDCM 00034]	> 10 yellow-brown colonies on CT-SMAC Agar	For all, > 10 yellow-brown colonies on CT-SMAC Agar <b>PASS</b>	For all, > 10 yellow-brown colonies on CT-SMAC Agar <b>PASS</b>
	Productivity / Qualitative	<i>Escherichia coli</i> ATCC® 25922™ [WDCM 00013] + <i>Staphylococcus aureus</i> ATCC® 25923™	> 10 pink colonies on CT-SMAC agar	> 10 pink colonies on CT-SMAC agar <b>PASS</b>	> 10 pink colonies on CT-SMAC agar <b>PASS</b>
	Selectivity / Qualitative	<i>Staphylococcus aureus</i> ATCC® 25923™ [WDCM 00034]	Total inhibition on Tryptic Soy Agar (TSA)	Total inhibition on TSA <b>PASS</b>	Total inhibition on TSA <b>PASS</b>

\*Additional strains used in the experiments of the study. Strains tested for mTSB were chosen by the QC laboratory.



## Selecting a water solution for media preparation

When preparing culture media by dissolving dehydrated media into water, it is important to select the correct water quality, since water may contain impurities that can affect media productivity or cause abnormalities such as incorrect pH, wrong color or precipitation, as indicated in Annex H of the EN ISO 11133 standard.<sup>1</sup> Water purified by the Milli-Q® IX water purification system fitted with a 0.22 µm final filter is validated to deliver water meeting or exceeding the water quality requirements of the EN ISO 11133 standard<sup>1</sup> (see **Appendix** for additional details). Other standards and methods, such as the American Public Health Association (APHA) Standard Methods,<sup>5</sup> make specific recommendations regarding water quality and purification technologies, to which the Milli-Q® IX system abides by its design and performance. Indeed, it includes, as recommended, a combination of technologies to produce pure water, resistivity monitoring, as well as a storage tank equipped with ultraviolet irradiation. The Milli-Q® IX system delivers water with a conductivity below 0.2 µS/cm, microbial levels below 10<sup>2</sup> cfu/mL, and free from traces of contaminants likely to inhibit the growth of microorganisms such as chlorine, ammonia and metals. Since the system delivers water that suits EN ISO 11133<sup>1</sup> water quality requirements, it will help to ensure the laboratory's regulatory compliance. However, it is important to also ensure that this water is well suited for the preparation of microbiological culture media.

The results from this study demonstrate that media prepared with water from a Milli-Q® IX system has equivalent characteristics and performance as the media prepared with the centrally purified DI water routinely used in the accredited QC laboratory. This indicates that water from this system can be used with confidence when preparing media according to the EN ISO 11133 standard.<sup>1</sup> Using a Milli-Q® IX water purification system offers many benefits to laboratories performing microbiology testing. According to the EN ISO 11133 standard,<sup>1</sup> water conductivity (or its reciprocal, resistivity) should be checked before use. The Milli-Q® IX water system offered the possibility to accurately measure water resistivity on-line and displayed it on the interface of the E-POD® dispenser, allowing a convenient monitoring solution. Checking water quality parameters was easy to perform via the intuitive touch screen compatible with gloves. The dispenser allowed time gain and precision as the flow rate could easily be adapted to the needs of the experiment, and the screen let the user program a specific volume to be dispensed. Moreover, the dispenser brought flexibility as it could be used over the sink or rested on its arm to fill larger containers. A foot pedal could have been added to facilitate serial dispensing while freeing the hands of the user. These features can

improve a lab's efficiency by simplifying scientists' working processes. The purified water delivered by the system can be used for other tasks, such as glassware rinsing, and to feed autoclaves or glassware washers, making the Milli-Q® IX system a comprehensive solution for microbiology laboratories.

Since water quality should not be overlooked when preparing media, the Milli-Q® IX system is designed to safeguard water purity. Water purified by the system is stored in a tank made of high-quality polyethylene, which doesn't release extractables into water and is protected against airborne contaminants by a vent filter. In addition, automatic recirculation of the pure water over a UVC LED lamp, as well as the 0.22 µm screen filter placed at the point-of-delivery of the system ensure reliably low microbial levels. Both the low level of extractables from the storage tank and the low microbial level in the water are important water quality parameters highlighted in the EN ISO 11133 standard.<sup>1</sup> In addition, thanks to the Elix® electrodeionization module,<sup>6</sup> the system delivers constant water quality, especially regarding conductivity, which is also one of the EN ISO 11133 quality requirements.<sup>1</sup> Using water of consistent quality to prepare media is an important factor in ensuring accurate and reproducible test results. This water, freshly produced in the laboratory, ensures direct control and autonomy.

Testing laboratories face many pressures, as they need to deliver results quickly in order to release lots in a timely manner, while at the same time ensuring the results are consistent, reproducible, accurate and reliable. Uncertainties regarding test results may cause scientists to troubleshoot and repeat experiments, which would cause delays and may postpone lot release, leading to financial loss. Having a reliable and independent source of purified water prevents any risk of unexpected changes in water quality or punctual unavailability of water from a central loop due to manufacturing constraints or maintenance.

Regular system maintenance is easily done by any member of the laboratory or can be delegated to a service engineer to ensure the system operates optimally. In addition, the system stores all data related to water quality parameters, as well as information such as consumable changes and other service activities, thus providing data traceability and paperless data management. This easy to retrieve data can simplify the laboratory's audit preparation. In addition, an online service is available to store all documentation and enable online service contract management. This allows to manage the water system more efficiently, in order to keep the laboratory working at maximum productivity.

## CONCLUSION

The study presented here shows that each of the media prepared with water from the Milli-Q® IX system successfully passed the performance tests according to the EN ISO 11133 standard<sup>1</sup> and gave similar outcomes as media prepared with the centrally produced DI water routinely used in the QC laboratory that regularly performs these tests. This demonstrates that, not only is the water quality delivered by this system compliant with the current version of EN ISO 11133,<sup>1</sup> but also that it has no impact on media performance and can be used with confidence to prepare microbiological culture media according to this standard.

In addition, the ease of use of the Milli-Q® IX system combined with its design features, such as the touchscreen displaying water quality parameters, data traceability and convenient dispensing options brings many benefits to the laboratory. Finally, the combination of water purification technologies inside the system, as well as the possibility to have a 0.22 µm filter at its point of dispense ensures that the water delivered is of constant quality, minimizing the risk of media performance issues caused by water. By combining high quality dehydrated media and water from a Milli-Q® IX purification system to prepare their media, scientists can optimize their processes, save time and gain in productivity. Obtaining both the dehydrated media and the water purification system from the same reputable supplier can bring added convenience and confidence.

## REFERENCES

1. EN ISO International Standardization Organization. Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media + Amendment 1 + Amendment 2. EN ISO 11133:2014/Amd1:2018/Amd2:2020.
2. ISO/IEC 17025:2017 - General requirements for the competence of testing and calibration laboratories.
3. ISO 7218:2007/Amd1:2013 - Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations.
4. ISO 8199:2018 - Water quality - General requirements and guidance for microbiological examinations by culture.
5. 9020 Quality assurance/Quality control; *Standard Methods for the Examination of Water and Wastewater*, 23<sup>rd</sup> Edition.; Washington, D.C.; American Public Health Association; 2017.
6. Dimitrakopoulos T, Feuillas E, Darbouret D, Mabic S. R&D Notebook 10. Electrodeionization: technology and applications. MilliporeSigma.

## Products related to this document:

Water purification systems and solutions	Article number
Milli-Q® IX 7003/05/10/15 Pure Water Purification System	<a href="#">ZIX7003P0</a>
Millipak® 0.22 µm filter	<a href="#">MPGP002A1</a>

  

Culture Media	Article number
Listeria agar (base) acc. OTTAVIANI and AGOSTI acc. ISO 11290	<a href="#">1004270500</a>
VRB (Violet Red Bile Lactose) agar acc. ISO 4832 and FDA-BAM	<a href="#">1014060500</a>
Malt extract agar	<a href="#">1053980500</a>
MRS (de MAN, ROGOSA and SHARPE) agar acc. ISO 15214	<a href="#">1106600500</a>
RVS (RAPPAPORT-VASSILIADIS-SOYA) broth (base) acc. ISO 6579	<a href="#">1007000500</a>
BRILA (Brilliant-green bile Lactose) broth acc. ISO 4831, ISO 4832 and FDA-BAM	<a href="#">1054540500</a>
mTSB broth with Novobiocin (20 mg/L) acc. ISO 16654	<a href="#">1092050500</a>



## APPENDIX

Compliance of the two types of water tested with EN ISO 11133<sup>1</sup> requirements.

	EN ISO 11133	DI water in the QC laboratory	Water from Milli-Q® IX system fitted with a 0.22 µm filter
Water purity	For the preparation of culture media, use only purified water, i.e. distilled, demineralized, deionized or produced by reverse osmosis, or of equivalent quality free from substances likely to inhibit or influence the growth of the microorganisms under the test conditions e.g. traces of chlorine, traces of ammonia and traces of metal ions.	Water is purified by a combination of reverse osmosis, ion-exchange and bactericidal UV lamp.	The system contains a combination of purification technologies: reverse osmosis, Elix® electrodeionization, bactericidal UVC LED, 0.22 µm filtration. Data obtained during the system's validation process: <ul style="list-style-type: none"> <li>• Free chlorine &lt; 0.02 ppm</li> <li>• Ammonia ≤ 0.00003%</li> <li>• Heavy metals ≤ 0.00001%</li> </ul>
Storage	The purified water shall be stored in tightly closed containers made from an inert material (neutral glass, polyethylene, etc.) which shall be free from all inhibitory substances. It is however recommended that the water is used as soon as produced.	Not applicable	Water is stored in a polyethylene tank, which is protected against airborne contaminants by a vent filter. An ergonomic E-POD® water dispenser provides easy delivery of pure water.
Microbial contamination	Microbial contamination should not exceed 10 <sup>3</sup> cfu/mL and preferably be below 10 <sup>2</sup> cfu/mL	Microbial contamination ≤ 10 <sup>2</sup> cfu/mL	Data obtained from an independent laboratory during the system's validation process: microbial contamination ≤ 10 <sup>2</sup> cfu/mL
Conductivity	The conductivity shall be no more than 25 µS/cm (equivalent to resistivity ≥ 0.04 MΩ cm), preferably below 5 µS/cm (grade 3 water, ISO 3696) at 25 °C. The conductivity should be checked before use.	Conductivity typically ≤ 2 µS/cm	System validated to deliver water with conductivity < 0.2 µS/cm; typically 0.1 µS/cm. The system is designed to meet or exceed requirements as described by EN ISO 3696, Grade 2 water. Last produced water resistivity is displayed on the instrument.

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