Millipore®
Preparation, Separation,
Filtration & Monitoring Products

Application Note

Comparison of Chromogenic Coliform Agar Membrane Filter Sets For Water Filtration Applications

In Compliance With ISO 9308-1:2014 and ISO 11133:2014

ISO 9308 part 1 (2014) specifies a detection method for *Escherichia coli* and coliform bacteria in water with a low bacterial background flora (e.g. drinking water and disinfected pool water). The method is based on membrane filtration and use of a chromogenic coliform agar medium.

The selective and differential properties of the chromogenic coliform agar (CCA) reduce testing time to 24 hours, from 48 hours with the previous Lactose TTC agar method.

The chromogenic substrates Salmon-GAL and X-glucuronide enable easy differentiation and enumeration of *Escherichia coli* and coliform bacteria, as both use characteristic enzyme combinations to cleave these substrates and grow as distinct coloured colonies. Enumeration of *Escherichia coli* includes all ß-D-galactosidase and ß-D-glucuronidase positive colonies (oxidase positive), which appear as dark-blue to violet. Enumeration of total coliforms includes all dark-blue and oxidase negative colonies with pink to red color.

ISO 11133 (2014) specifies a method to test culture media used for membrane filtration. Following filtration of a defined inoculated liquid, the membrane filter is placed on the surface of the tested agar. The productivity of the agar-membrane filter set is calculated compared to a reference media. The process must be repeated after every change of the membrane filter batch and /or agar batch, considerably increasing the workflow and costs for the customer.

We offer certified ReadyPlate™ 55 KIT CCA, which contains ready to use 55 mm media plates and EZ-Pak® Membrane Filters. ReadyPlate™ 55 KIT CCA is compliant to ISO 11133 (2014) and ISO 9308 part 1 (2014), thereby reducing customer quality control costs for membrane filter and agar batch testing.

The scope of this comparison study was to evaluate the productivity performance of the chromogenic coliform agar-membrane filter sets, which are currently available on the market.

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Material and Equipment:

Table 1. Media Membrane Filter Sets

Product Name	Cat. No.	Format	ISO 9308-1:2014 compliant
ReadyPlate™ 55 CCA	**1.46757.0020**	**Ready-to-Use**	**Yes**
Company A	-	Semi-Dehydrated Pad*	No
Company B	-	Semi-Dehydrated Pad*	No

* Pad contains nutrients. Requires rehydration before use (3-3.5 mL)

We offer ready-to-use membrane filter sets (ReadyPlate™ 55 KIT CCA) compliant to ISO 9308 part 1 (2014). Our mixed cellulose ester filters (gridded, 0.45 µm pore size, EZ-Pak®, cat. no. EZHAWG474) were used in ISO validation studies for chromogenic coliform agar (Lange et al. 2013).

The matched membrane were used in this comparison study.

Table 2. Matched Membrane Filter by the Manufacturer

Product Name	Cat. No.	Filter Type	Pore size	Diameter
ReadyPlate™ 55 CCA	**EZHAWG474**	**Mixed Cellulose Ester**	**0.45 µm**	**47 mm**
Company A	-	Mixed Cellulose Ester	0.45 µm	50 mm
Company B	-	Cellulose Nitrate	0.45 µm	47 mm

An incoming inspection on specific criteria was performed. (Table 3)

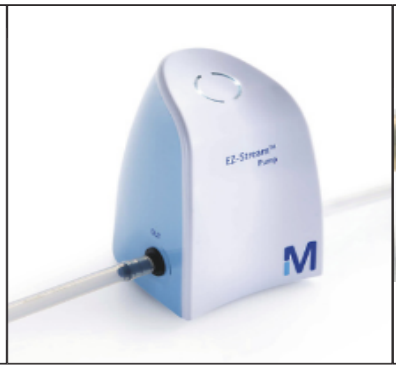
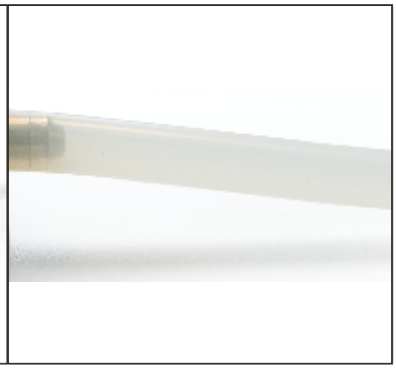
Table 3. Inspection (ReadyPlate™ 55 CCA, Company A and Company B)

	ReadyPlate™ 55 CCA	Company A / Company B
ISO Compliance	**Yes**	**No**
ISO 17025 released (for kits)	**Yes**	**No**
Lockable Plates	**Yes**	**No**
Stackable Plates	**Yes**	**No**
Directly usable	**Yes**	**No** (Requires dehydratation)
Room Temperature Storage	**Yes**	**Yes**
Shelf Life	**9 month**	**2 years**

Table 4. Reference Plate & Liquids

Product Name	Cat. No.	Usage
Tryptic Soy Agar (TSA) – LI 30 mL EP+USP	**1.46004.0020**	Reference Plate
0,9 % NaCl Solution (Milli-Q® water)	**Sodium Chloride (1.06400.1000)**	Filtration
Sodium Chloride Peptone broth (buffered)	**1.10582.0500**	Dilution

Table 5. Equipment. Microfil® funnels for filtration

EZ-Fit® Manifold, 6-place for Microfil® Funnels and membranes	EZ-Stream® Vacuum pump	Silicon hose - autoclavable
EZFITMIC06	EZSTREAM1	STREAMTUB
		

Method:

The productivity test organisms were selected according to ISO 9308 part 1 (2014).
Recovery rate criteria, rather than productivity rates are listed in Table 6.

Table 6. Performance criteria and productivity control strains according to ISO 9308-1:2014 (table 1) The recovery rate of 70 % is equivalent to a productivity value of 0.7.

Function	Incubation	Control strains	Reference medium	Method of control	Criteria (Recovery rate)	Characteristic reactions
Productivity	21 hours (± 3) at 36 °C (± 2)	*Escherichia coli* WDCM 00013 (ATCC 25922) *Escherichia coli* WDCM 00012 (ATCC 8739)	Tryptic Soy Agar (TSA)	Quantitative with membrane filtration	70 %	Dark-blue to violet colonies
	21 hours (± 3) at 36 °C (± 2)	*Enterobacter aerogenes* WDCM 00175 (ATCC 13048) *Citrobacter freundii* WDCM 00006 (ATCC 43864)	Tryptic Soy Agar (TSA)	Quantitative with membrane filtration	70 %	Pink to red colonies

Figure 1 illustrates the handling workflow according to the manufacturers' instruction.

ReadyPlate™ 55 CCA is a ready-to-use agar plate.

Companies A and B are semi-dehydrated pad products requiring an initial rehydration step.

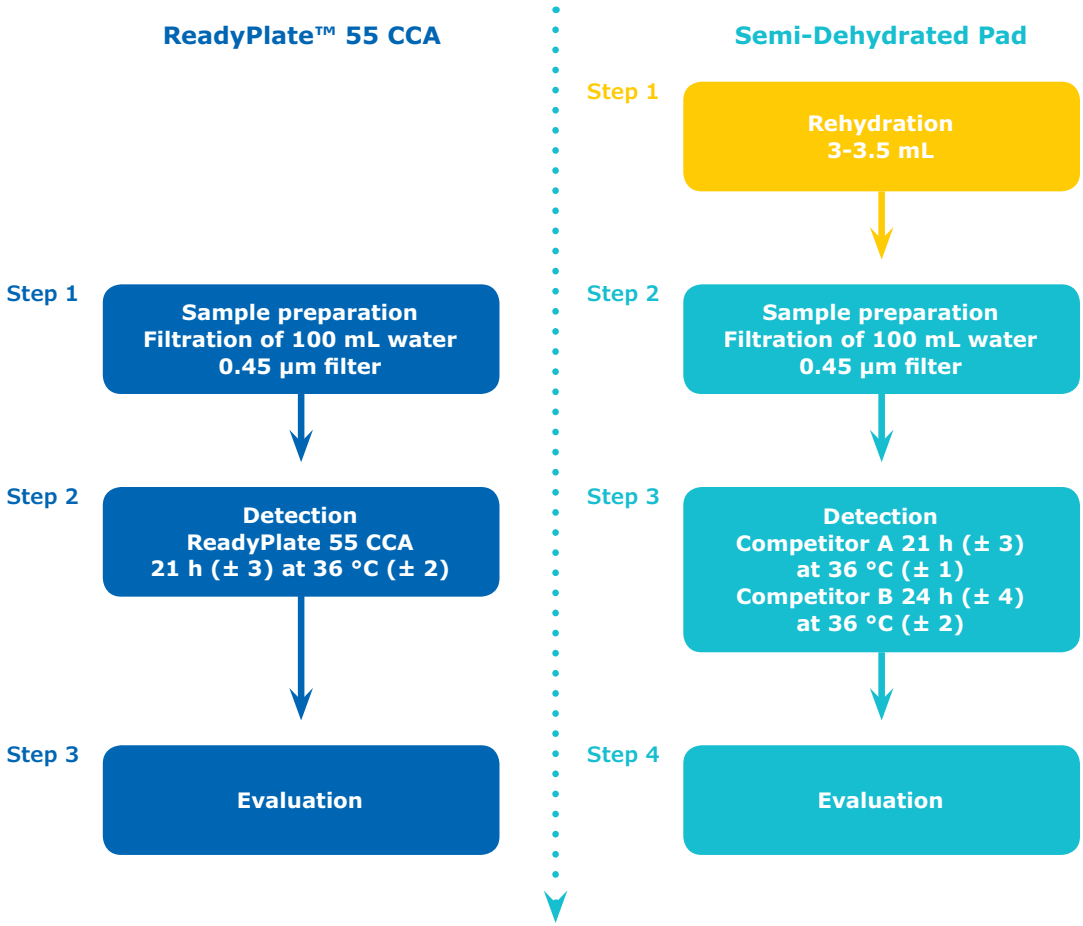


Figure 1.
Workflow ready-to-use ReadyPlate™ 55 CCA and semi-dehydrated pad (Rehydration step necessary) to detect low bacteria background in water with membrane filtration method.

The performance of CCA was tested according to the methods and criteria described in ISO 11133 (2014).

Application Comparison Study:

- 100 mL 0.9 % sodium chloride solution was inoculated with the test organism
- Sample was filtered using the matched membrane filter.
- Membrane filter was placed onto CCA. Application of the membrane filter was controlled to prevent formation of air bubbles underneath.
- ReadyPlate™ 55 CCA plates were inverted for incubation. Semi-dehydrated pads were incubated with lid uppermost.

According to ISO 11133 (2014) the shortest incubation time should be used for target organisms.

Table 7. Incubation Time Comparison Study

Product	Incubation Time
ReadyPlate™ 55 CCA	**18 h**
Company A	18 h
Company B	20 h*

***Minimum incubation according to the manufacturer's specification.**

- Evaluation was initiated immediately on completion of the incubation time.
- Enumeration was conducted according to the characteristic criteria in ISO 9308-1:2014
- Productivity rate was calculated according to the mathematical formula in ISO 11133 (2014) (see Note right).

Note:

The productivity of a culture medium is the level of recovery of target organism under defined condition (ISO 11133:2014 Section 3.2.4).

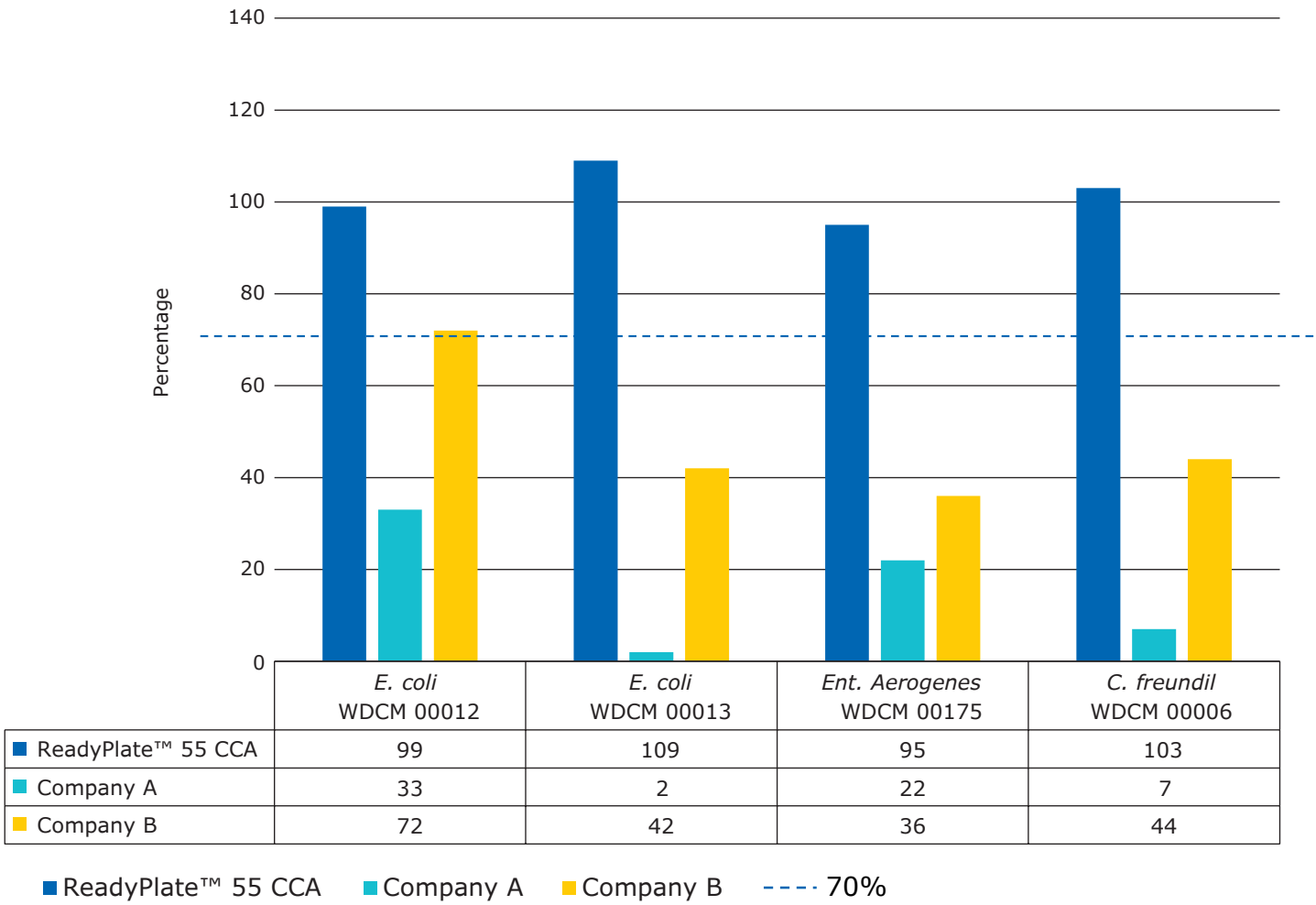
For quantitative methods the productivity ratio (PR) is determined:

$$P_R = \frac{\text{Total count of colonies obtained on the culture medium under test (Membrane filter on CCA)}}{\text{Total count of colonies obtained on defined reference medium (Direct inoculation on TSA)}}$$

Results:

- ReadyPlate™ 55 CCA plates are purposely designed to contain agar up to the top of the plate to greatly improve membrane filter application and removal. It was also observed that when used in combination with the EZ-Pak® membrane filters, a perfect contact between media and membrane filter was achieved.
- The semi-dehydrated pad must be rehydrated before use. The pads should be pre-wet with a volume of 3.0-3.5 mL. It was observed that the correct volume is important.
- Too small a volume prevents the membrane from lying flat on the pad, reducing the nutrient supply in these positions. When an optimal nutrient supply for micro-organisms is not provided, there is a risk of obtaining false negative results.
- Too much water increases the possibility of water flowing onto the membrane filter, causing colonies to merge, appear indistinct and thereby difficult to identify.
- The bar chart (Figure 2) illustrates the recovery rate of the productivity performance of the different products and test organisms. ReadyPlate™ 55 CCA results are listed first, followed by Companies A and B.

Productivity CCA Membrane Filter Sets



	E. coli WDCM 00012	*E. coli* WDCM 00013	*Ent. Aerogenes* WDCM 00175	*C. freundil* WDCM 00006
ReadyPlate™ 55 CCA	99	109	95	103
Company A	33	2	22	7
Company B	72	42	36	44

Figure 2.
Recovery rate [%] of the comparison study CCA Membrane Filter Sets (ReadyPlate™ 55 Kit CCA, Company A and Company B). Recovery rate criteria for CCA according to ISO 9308-1:2014 is ≥ 70 %.

Recovery rate criteria according to ISO 9308 part 1 (2014) is ≥ 70 %. Performance criteria was reached for our ReadyPlate™ 55 CCA and all recovery rates are clearly above the determined value.

Table 8. Recovery rate in percentage [%]

	Escherichia coli WDCM 00012	*Escherichia coli* WDCM 00013	*Enterobacter aerogenes* WDCM 00175	*Citrobacter freundii* WDCM 00006
ReadyPlate™ 55 CCA	**99**	**109**	**95**	**103**
Company A	33	2	22	7
Company B	72	42	36	44

- The comparison study demonstrated, that the recovery rate of company A's semi-dehydrated pad set is significantly below the defined specification of ISO 9308 part 1 and significantly different to ReadyPlate™ 55 Kit CCA.
- Company B reached the specification only with one organism, *Escherichia coli* WDCM 00012.

The recovery rate of the second *Escherichia coli* strain and the two coliform bacteria (Enterobacter aerogenes and Citrobacter freundii) are far below the criteria of ISO 9308 part 1.

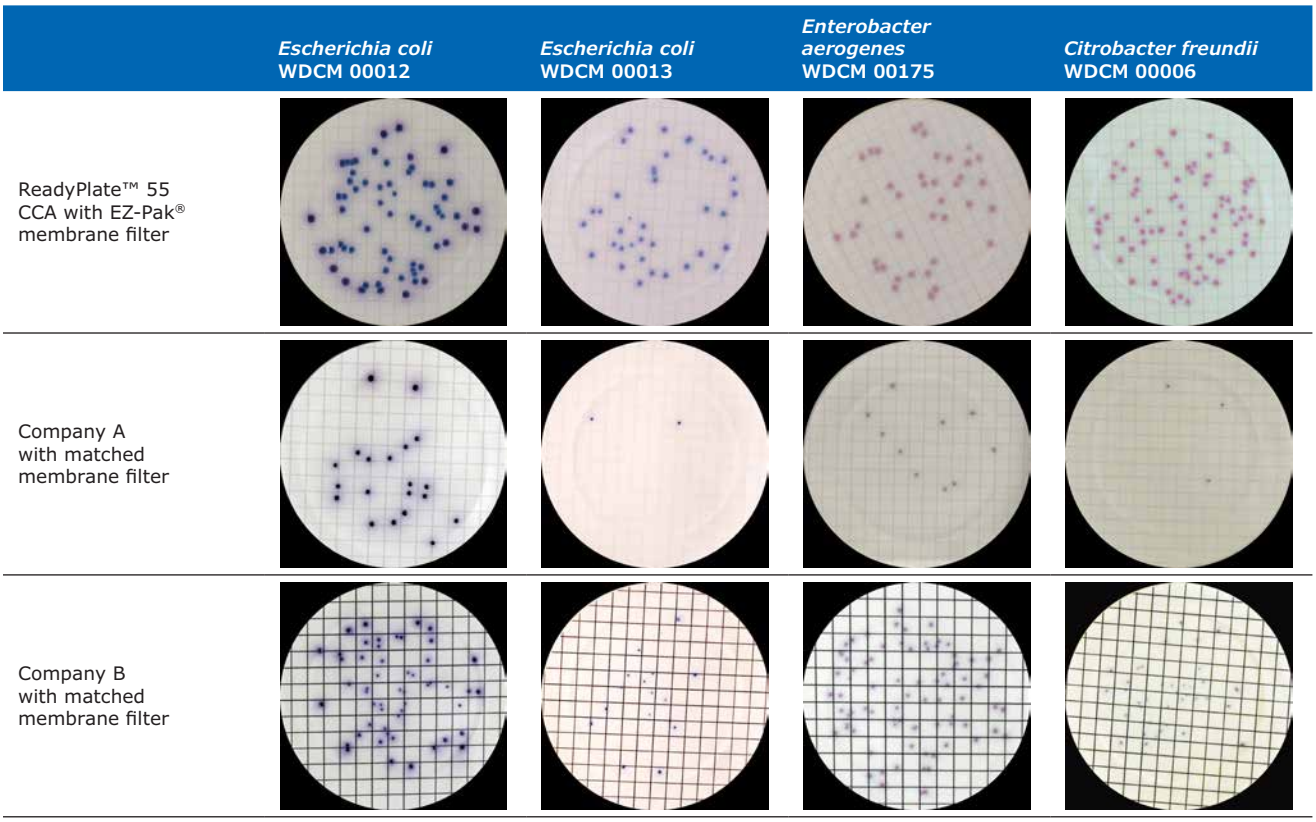


Figure 3. Photo ReadyPlate™ 55 CCA and companies A and B with membrane filter.

- EZ-Pak® membrane filter did not have a negative influence on colony size with ReadyPlate™ 55 CCA.
- However, different colony sizes were observed with the matched company B membrane filter on the semi-dehydrated pad.

Interpretation:

ReadyPlate™ 55 CCA membrane filter sets easily attained the productivity criteria of ≥ 70 % according to ISO 9308 part 1 (2014) with all control strains.

ReadyPlate™ 55 CCA plates can be used in combination with EZ-Pak® Membrane Filter to enumerate E. coli and coliform bacteria in water according to ISO 9308-1:2014 standard.

The productivity performance of company A did not reach the defined ISO 9308 part 1 (2014) specification and is unsuitable to test water.

Company B only reached the criteria for *Escherichia coli* WDCM 00012, but not for all the other control strains.

ReadyPlate™ 55 CCA membrane filter sets save customers time and cost. The incoming QC tests for membrane batch with agar batch combination are optimized.

ReadyPlate™ 55 KIT CCA ISO 9308 is ISO 11133 compliant and released through an ISO 17025 accredited laboratory, enabling end-users to reduce incoming QC.

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