Microbiological Quality of Non-sterile Products

Culture Media for Compendial Methods

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

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Preparation, Separation, Filtration & Testing Products

Culture Media for Compendial Methods

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When determining whether a substance or preparation complies with established microbiological quality, it is important to use the correct media. The three internationally most important Pharmacopoeias (EP/JP/USP) published harmonized versions of their chapters on microbial examination of non-sterile products in 2009. In addition, the 2016 edition of the Chinese Pharmacopoeia is, for the most part, harmonized in terms of media composition and performance criteria, although growth promotion testing is required to be performed with strains from the Chinese strain collection. These methods address the total aerobic count (TAMC and TYMC) as well as the test for specified microorganisms.

MilliporeSigma's culture media portfolio for microbial examination of non-sterile products is fully compliant with the harmonized Pharmacopoeia. As compendial methods change, be assured that we monitor this thoroughly, and any changes to composition or performance testing will be implemented quickly so that you won't need to worry about the regulatory compliance of our media.

MilliporeSigma provides a choice of products for all culture media and substances required for

- Sample Preparation
- Microbial Enumeration Tests
- Tests for Specified Microorganisms

European Directorate for the Quality of Medicines and Healthcare. (2017): The European Pharmacopoeia. 9th Ed. Chapter 2.6.12 Microbiological examination of non-sterile products: Microbial enumeration tests and Chapter 2.6.13 Microbiological examination of non-sterile products: Test for specified products. Strasbourg, France.

Japanese Ministry of Health, Labour and Welfare. (2016): The Japanese Pharmacopoeia. 17th Ed. Chapter 4.05 Microbial Limit Test I. Microbiological examination of non-sterile products: Total viable aerobic count and II. Microbiological examination of non-sterile products: Test for specified products. Japanese Ministry of Health, Labour and Welfare. Tokyo, Japan.

United States Pharmacopeial Convention. (2014): The United States Pharmacopeia 38/National Formulation 33, Supp. 2. Chapter <61> Microbiological examination of non-sterile products: Microbial enumeration tests and Chapter <62> Microbiological examination of non-sterile products: Test for specified microorganisms. Rockville, Md., USA.

Preparation of the Sample

Depending on the properties of the products to be tested, there are choices of dilution media and additives.

Table 1: Sample preparation

Medium / Substance	Intended Use
Buffered Sodium Chloride Peptone Solution pH 7.0 **Phosphate Buffer Solution pH 7.2** **Soybean Casein Digest Broth**	Diluents for all non-fatty products, soluble or insoluble in water
Polysorbate 80	Products insoluble in water, added to assist the suspension of substances with poor wettability Fatty products
Isopropyl myristate	Fatty products
Lecithin	Transdermal patches

Many products bear antimicrobial properties which may be countered by adding neutralizing agents. The following agents are suggested by Pharmacopoeia

Table 2: Neutralizing agents for interfering substances

Neutralizing Agents	Interfering Substances
Sodium Hydrogen Sulfite	Glutaraldehyde, mercurials
Glycine	Aldehydes
Lecithin	Quaternary ammonium compounds (QACs), parahydroxybenzoates (parabens), bis-biguanides
Polysorbate	QACs, iodine, parabens
Thioglycollate	Mercurials
Thiosulfate	Mercurials, halogens, aldehydes

Microbial Enumeration Tests

Microbial Enumeration Tests are quantitative methods to determine a product's level of contamination with mesophilic bacteria and fungi that grow under aerobic conditions.

The tests are comprised of total aerobic microbial count (TAMC) and total yeast and mold count (TYMC).

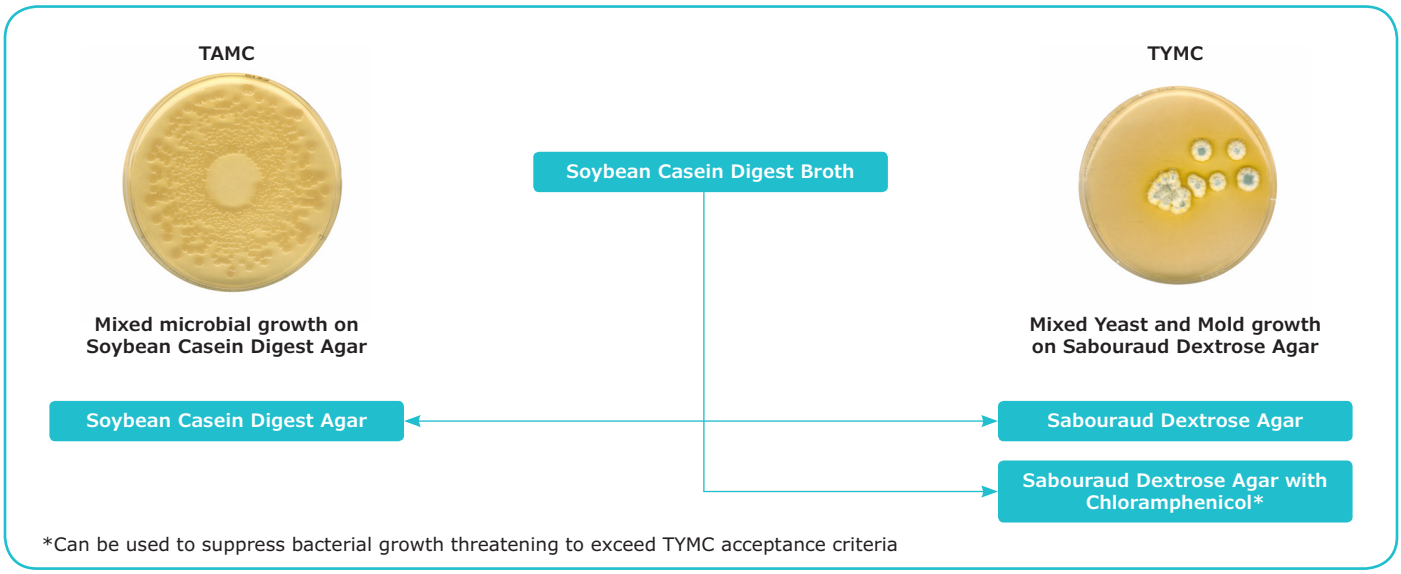
Depending on the product to be tested, the most appropriate enumeration method can be chosen from Membrane Filtration, Plate-Count methods, or the Most-Probable-Number (MPN) method.

Before a method is chosen, the suitability needs to be proven in the presence of the product. This involves the preparation of suitable test strains, growth promotion tests of the culture media, as well as suitability tests for the counting method in the presence of the product.

Table 3: Sample preparation

Preparation of Test Strains	Preparation of Test Strains	Growth Promotion	Suitability of Counting Method in the Presence of Product
Staphylococcus aureus	Soybean Casein Digest Agar or Soybean Casein Digest Broth	Soybean Casein Digest Agar or Soybean Casein Digest Broth	Soybean Casein Digest Agar (MPN) or Soybean Casein Digest Broth
Pseudomonas aeruginosa	Soybean Casein Digest Agar or Soybean Casein Digest Broth	Soybean Casein Digest Agar or Soybean Casein Digest Broth	Soybean Casein Digest Agar (MPN) or Soybean Casein Digest Broth
Bacillus subtilis	Soybean Casein Digest Agar or Soybean Casein Digest Broth	Soybean Casein Digest Agar or Soybean Casein Digest Broth	Soybean Casein Digest Agar (MPN) or Soybean Casein Digest Broth
Candida albicans	Sabouraud Dextrose Agar or Sabouraud Dextrose Broth	Soybean Casein Digest Agar (TAMC) Sabouraud Dextrose Agar (TYMC)	Soybean Casein Digest Agar (TAMC) Sabouraud Dextrose Agar (TYMC)
Aspergillus brasiliensis	Sabouraud Dextrose Agar or Potato Dextrose Agar and Polysorbate 80 (optional)	Soybean Casein Digest Agar (TAMC) Sabouraud Dextrose Agar (TYMC)	Soybean Casein Digest Agar (TAMC) Sabouraud Dextrose Agar (TYMC)

Diagram: Microbial Enumeration Tests



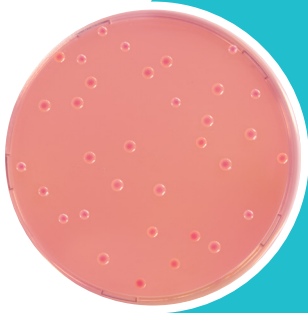
Tests for Specified Microorganisms

Depending on the microbiological quality specification of each non-sterile pharmaceutical product, the manufacturer is required to determine the absence or limited occurrence of the following seven organisms:

1. Bile-Tolerant Gram-Negative Bacteria
2. *Escherichia coli*
3. *Salmonella*
4. *Pseudomonas aeruginosa*
5. *Staphylococcus aureus*
6. Clostridia
7. *Candida albicans*

These organisms serve as general indicator organisms, but depending on the nature of the product, its designated use, the recipient group and method of application, other pathogenic microorganisms may be relevant. The following overview of required media is limited to explicit Pharmacopoeia requirements.

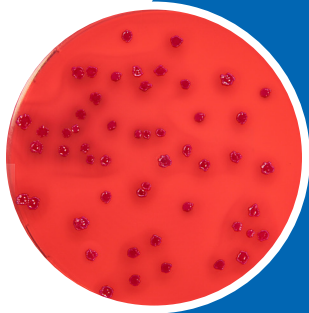
Test for Specified Microorganisms



Organism:
Bile-Tolerant Gram-Negative Bacteria

Dilution / Enrichment:
Soybean Casein Digest Broth
Enterobacteria Enrichment Broth Mossel

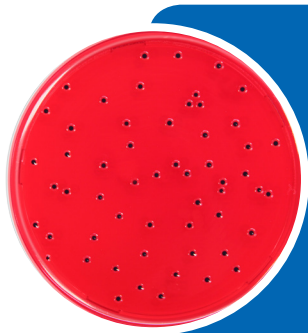
Subcultivation:
Violet Red Bile Glucose Agar



Organism:
Escherichia coli

Dilution / Enrichment:
Soybean Casein Digest Broth
MacConkey Broth

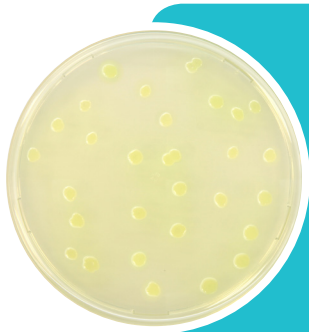
Subcultivation:
MacConkey Agar



Organism:
Salmonella

Dilution / Enrichment:
Soybean Casein Digest Broth
Rappaport-Vassiliadis Salmonella Enrichment Broth

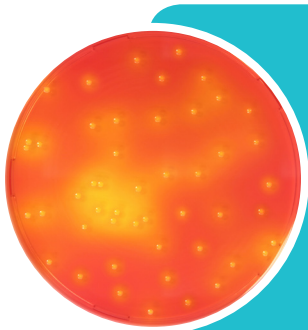
Subcultivation:
Xylose Lysine Deoxycholate Agar



Organism:
Pseudomonas aeruginosa

Dilution / Enrichment:
Soybean Casein Digest Broth

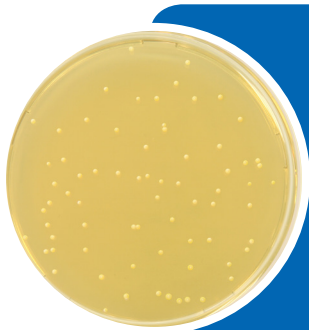
Subcultivation:
Cetrimide Agar



Organism:
Staphylococcus aureus

Dilution / Enrichment:
Soybean Casein Digest Broth

Subcultivation:
Mannitol Salt Agar



Organism:
Candida albicans

Dilution / Enrichment:
Sabouraud Dextrose Broth

Subcultivation:
Sabouraud Dextrose Agar

Organism:
Clostridia

Dilution / Enrichment:
Reinforced Medium for Clostridia

Subcultivation:
Columbia Agar

Ordering Information

Medium / Substance	Catalogue No.	Format / Packaging	Package Size
Buffered Sodium Chloride Peptone Solution pH 7.0	**1.10582.0500**	dehydrated, plastic bottle	**500 g**
	1.10582.5000	dehydrated, plastic drum	**5 kg**
	1.46196.0100	ready-to-use, 9 mL in 17 mL glass tube with red cap	**100 pcs**
	1.46141.0020	ready-to-use, 9 mL in 17 mL glass tube with blue cap	**20 pcs**
	1.46480.0010	ready-to-use, 90 mL in 125 mL glass bottle with blue screw cap and three loci	**10 pcs**
	1.46407.0006	ready-to-use, 90 mL in 275 mL wide mouth glass bottle	**6 pcs**
	1.46367.0010	ready-to-use, 100 mL in 125 mL glass bottle with blue screw cap and 3 loci	**10 pcs**
	1.46369.0010	ready-to-use, 100 mL in 125 mL glass bottle with blue flip cap and septum	**10 pcs**
	1.46360.0006	ready-to-use, 90 mL in 250 mL glass bottle with blue screw cap and 3 loci	**6 pcs**
	1.46419.0006	ready-to-use, 200 mL in 250 mL glass bottle with blue screw cap and 3 loci	**6 pcs**
	1.46611.0006	ready-to-use, 200 mL in 250 mL glass bottle with blue flip cap and septum	**6 pcs**
	1.46408.0006	ready-to-use, 300 mL in 500 mL glass bottle with blue flip cap and septum	**6 pcs**
	1.46585.0006	ready-to-use, 600 mL in 1 L glass bottle with screw cap and 3 loci	**6 pcs**
	1.46368.0006	ready-to-use, 1 L in 1 L glass bottle with blue screw cap and 3 loci	**6 pcs**
Cetrimide Agar	**1.05284.0500**	dehydrated, plastic bottle	**500 g**
	1.46048.0020	ready-to-use 30 mL plates	**20 pcs**
	1.46048.0120	ready-to-use 30 mL plates	**120 pcs**
Columbia Agar	**1.10455.0500***	dehydrated, plastic bottle	**500 g**
	1.10455.5000*	dehydrated, plastic drum	**5 kg**
Enterobacteria Enrichment Broth Mossel	**1.05403.0500**	dehydrated, plastic bottle	**500 g**
	1.46463.0010	ready-to-use, 100 mL broth in 125 mL glass bottle	**10 pcs**
	1.46219.0020	ready-to-use, 10 mL broth in 17 mL glass tube with yellow cap	**20 pcs**
	1.46219.0100	ready-to-use, 10 mL broth in 17 mL glass tube with yellow cap	**100 pcs**
Glycine	**1.04201.0100**	plastic bottle	**100 g**
	1.04201.0250	plastic bottle	**250 g**
	1.04201.1000	plastic bottle	**1 kg**
	1.04201.5000	plastic bottle	**5 kg**
Isopropyl Myristate	**8.17066.2500***	glass bottle	**2.5 L**
	1.46628.0006**	360 mL glass bottle, gamma-irradiated at 25 kGy	**6 pcs**
Lecithin	**4.29415-100GM**	glass bottle	**100 g**
Mannitol Salt Agar	**1.05404.0500**	dehydrated, plastic bottle	**500 g**
	1.05404.5000	dehydrated, plastic drum	**5 kg**
	1.46023.0020	ready-to-use 30 mL plates	**20 pcs**
	1.46023.0120	ready-to-use 30 mL plates	**120 pcs**

Medium / Substance	Catalogue No.	Format / Packaging	Package Size
MacConkey Agar	**1.05465.0500**	dehydrated, plastic bottle	**500 g**
	1.05465.5000	dehydrated, plastic drum	**5 kg**
	1.46022.0020	ready-to-use 30 mL plates	**20 pcs**
	1.46022.0120	ready-to-use 30 mL plates	**120 pcs**
MacConkey Broth	**1.05396.0500**	dehydrated, plastic bottle	**500 g**
	1.46382.0006	ready-to-use, 1 L in 1 L glass bottle with blue screw cap and 3 loci	**6 pcs**
	1.46372.0006	ready-to-use, 100 mL in 250 mL glass bottle with blue screw cap and 3 loci	**6 pcs**
	1.46461.0010	ready-to-use, 100 mL in 125 mL glass bottle	**10 pcs**
	1.46383.0006	ready-to-use, 100 mL in 275 mL wide mouth glass bottle	**6 pcs**
Phosphate Buffer Solution pH 7.2	**1.46777.0006 **/*** **	ready-to-use, 500 mL glass bottle	**6 pcs**
Polysorbate 80	**8.17061.1000**	plastic bottle	**1 L**
	8.17061.2500	plastic bottle	**2.5 L**
Potato Dextrose Agar	**1.10130.0500**	dehydrated, plastic bottle	**500 g**
	1.10130.5000	dehydrated, plastic drum	**5 kg**
Rappaport Vassiliadis Salmonella Enrichment Broth	**1.07666.0500**	dehydrated, plastic bottle	**500 g**
	1.46181.0020	ready-to-use, 10 mL in 17 mL tube with yellow cap	**20 pcs**
	1.46181.0100	ready-to-use, 10 mL in 17 mL tube with yellow cap	**100 pcs**
Reinforced Medium for Clostridia	**1.05411.0500**	dehydrated, plastic bottle	**500 g**
Sabouraud Dextrose Agar	**1.05438.0500**	dehydrated, plastic bottle	**500 g**
	1.05438.5000	dehydrated, plastic drum	**5 kg**
	1.46028.0020	ready-to-use 30 mL plates	**20 pcs**
	1.46028.0120	ready-to-use 30 mL plates	**120 pcs**
Sabouraud Dextrose Agar with Antibiotics	**1.00918.0500**	dehydrated, plastic bottle	**500 g**
	1.46003.0020	ready-to-use 30 mL plates	**20 pcs**
	1.46003.0120	ready-to-use 30 mL plates	**120 pcs**
Sabouraud Dextrose Broth	**1.08339.0500**	dehydrated, plastic bottle	**500 g**
	1.46366.0010	ready-to-use, 100 mL in 125 mL glass bottle	**10 pcs**
Sodium Hydrogen Sulfite	**8.45119.0100**	plastic bottle	**100 mL**
	8.45119.1000	plastic bottle	**1 L**
Soybean Casein Digest Agar (TSA)	**1.05458.0500**	dehydrated, plastic bottle	**500 g**
	1.05458.5000	dehydrated, plastic drum	**5 kg**
	1.05458.9010	dehydrated, plastic drum	**10 kg**
	1.46004.0020	ready-to-use 30 mL plates	**20 pcs**
	1.46004.0120	ready-to-use 30 mL plates	**120 pcs**
Soybean Casein Digest Broth (TSB)	**1.05459.0500**	dehydrated, plastic bottle	**500 g**
	1.05459.5000	dehydrated, plastic drum	**5 kg**
	1.05459.9010	dehydrated, plastic drum	**10 kg**
	1.05459.9025	dehydrated, plastic bag in cardboard box	**25 kg**
	1.46458.0010	ready-to-use, 100 mL in 125 mL glass bottle	**10 pcs**
Thioglycollate (Sodium Thioglycolate)	**1.06691.0500**	glass bottle	**500 g**
Thiosulfate (Sodium Thiosulfate)	**1.06512.0250**	plastic bottle	**250 g**
	1.06512.2500	plastic bottle	**2.5 kg**
Violet Red Bile Glucose Agar	**1.10275.0500**	dehydrated, plastic bottle	**500 g**
	1.46000.0020	ready-to-use 30 mL plates	**20 pcs**
	1.46000.0120	ready-to use 30 mL plates	**120 pcs**
Xylose Lysine Deoxycholate Agar	**1.05290.0500**	dehydrated, plastic bottle	**500 g**
	1.46073.0020	ready-to-use 30 mL plates	**20 pcs**
	1.46073.0120	ready-to-use 30 mL plates	**120 pcs**

(*) not available in North America
(**) Customized product, please inquire about availability.
(***) Please note that the composition of this Phosphate Buffer Solution is slightly different from the compendial suggestion

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