Pharmaceuticals

Pharmaceutical Microbiology

Science beyond expectations

Contents

Introduction

Pharmaceutical industries based on their manufacturing pattern can be broadly classified into the following categories:

1. **Sterile pharma and biopharma**
   Injectables, sterile ophthalmic drops, ointments, monoclonal antibodies (MAbs) and vaccines

2. **Non-sterile pharma**
   Tablets, capsules, ophthalmic drugs

Sterile: Absence of microbes in a product.

Sterility testing

The testing which confirms the absence of microbial presence in the products is known as sterility testing. Sterile pharmaceutical ingredients, medical devices and materials claimed to be sterile undergo strict sterility testing to ensure they adhere to regulations. The sterile products are produced in the cleanroom environment free from any microbial contamination.

The testing methods which ensure the product sterility state that a positive result (microbial growth) after 14 days at appropriate temperature (7 days incubation at 20 °C - 25 °C followed by 7 days incubation at 30 °C - 35 °C) is considered a fail, whereas negative results shows that the product is sterile. The sterile products can be produced either by following aseptic manufacturing techniques or terminal sterilization.

Non-sterile

Unlike sterile pharma manufacturing, non-sterile pharma products do not require the cleanroom environment to be completely free from any microbial presence.

Pharma manufacturing workflow

Pharmacopeia

The term “Pharmacopeia” has been derived from Greek words “pharmakon”, which means drug, and “poiein”, meaning to make. Thus, Pharmacopeia is a manual which provides information on drug manufacturing.

Harmonized Pharmacopeia

- United States Pharmacopeia (USP)
- European Pharmacopeia (EP)
- Japanese Pharmacopeia (JP)

To streamline the process of drug manufacturing across the globe, the above mentioned three pharmacopeias have been harmonized to form the “Harmonized Pharmacopeia”.

Apart from the aforesaid Pharmacopeia, there are numerous other pharmacopeia globally which vary from one country to another. Among these, British Pharmacopeia (BP) is also widely accepted among the commonwealth countries, which comprises 54 countries, and a combined GDP estimated to reach $13 trillion in 2020. Similarly, China and India have separate Pharmacopeia known as Chinese & India Pharmacopeia (CP & IP), respectively.

Overview of key markets

Drug export market and key players

During 2019, the total business from drug and medicines from export was USD 392.9 trillion.

The highest dollar value of business registered by different countries is highlighted on the right-hand side, with percentage of share. The tally is lead by Germany, which exported USD 56.9 billion worth of medicines and drugs in FY 2019.

Similarly, for FY 2019, the United States topped the chart in drug imports from other countries. China, India and Mexico sell maximum drug and medicines in US market.

No.	Importer countries	Revenue with % share
1.	United States	$78.9 billion (18.7% of imported drugs/medicines)
2.	Germany	$30.9 billion (7.3%)
3.	Belgium	$24 billion (5.7%)
4.	China	$21.5 billion (5.1%)
5.	Switzerland	$21.3 billion (5%)
6.	United Kingdom	$18.8 billion (4.5%)
7.	Netherlands	$17.5 billion (4.2%)
8.	Italy	$17.3 billion (4.1%)
9.	Japan	$17 billion (4%)
10.	France	$15.8 billion (3.8%)
11.	Spain	$11 billion (2.6%)
12.	Russia	$10.2 billion (2.4%)
13.	Canada	$8.3 billion (2%)
14.	Australia	$5.3 billion (1.3%)
15.	Poland	$5.2 billion (1.2%)

1. Germany
$56.9 billion (14.5%)

2. Switzerland
$47.8 billion (12.2%)

3. Netherlands
$31.1 billion (7.9%)

4. Belgium
$29 billion (7.4%)

5. France
$26.2 billion (6.8%)

6. Italy
$24.8 billion (6.3%)

7. United States
$24.3 billion (6.2%)

8. United Kingdom
$18.3 billion (4.7%)

9. Ireland
$17.9 billion (4.6%)

10. Denmark
$15.5 billion 4%)

11. India
$14.8 billion (3.8%)

12. Spain
$10.1 billion (2.6%)

13. Sweden
$8.2 billion 2.1%)

14. Canada
$7.5 billion (1.9%)

15. Austria
$.8 billion (1.5%)

Sterility of the media

There are mainly two types of media that are used in sterility:

a. Fluid Thioglycollate Medium (FTM)

b. Tryptone Soya Broth or Soyabean Casein Digest Medium (TSB or SCDM)

The composition of the media used for sterility testing must comply to the Harmonized Pharmacopeia (USP/EP/JP).

Sterility check for the media

This is a pre-requisite step, which states that the sterility testing medium must not contain any contamination. To ensure the media is sterile (free from any microbial contamination), prepare FTM and TSB media and incubate the same at appropriate temperatures for a total of 14 days. There should not be any turbidity in the media after the said period.

Dehydrated culture media sterility for 14 days

Dehydrated culture medium

In-house preparation

14 days

Incubator

Pass
Clear medium

Failed
Turbidity/hazy medium

Ready-to-use media or PPM sterility testing for 14 days

In-house preparation

Incubator

Pass
Clear medium

Failed
Turbidity/hazy medium

Growth promotion test (GPT)

GPT for media as per harmonized Sterility Tests

Each lot of media undergoes a growth promotion test, regardless of whether it is prepared or dehydrated culture media. In a growth promotion test, the media is inoculated with <100 CFU of the mentioned Quality Control (QC) organisms.
Clostridium sporogenes, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* organisms are inoculated in Fluid Thioglycollate Medium and incubated at 30 °C - 35 °C for 3 days.

Similarly, *Aspergillus brasiliensis, Bacillus subtilis*, and *Candida albicans* are inoculated in Tryptone Soya Broth at 20 °C - 25 °C for 5 days (fungi) & 20 °C - 25 °C for 3 days (bacteria).

The media are suitable if a clearly visible growth occurs.

There are mainly two types of culture strains that are available in the market; crude cultures, which are required to undergo serial dilution prior to the use, and commercially-available, ready-to-use culture strains. As per the regulatory-set standards the culture strain standards should not exceed the fifth passage or sub-cultures.

Please refer to the non-sterility growth-promotion section for the workflow.

Method of suitability

The method for suitability test ensures that any antimicrobial agent present in the product can be effectively neutralized or inhibited and there will be no microbial growth inhibition due to the same. This is an important test for new products and/or change/modification made in the original experimental set-up. This test ensures that if products contain contamination, it can be grown in suitable media in order to seize the release of contaminated product(s).

Suitability test can be performed by:

1. Membrane filtration method

As per USP <71>, EP <2.6.1> & JP <4.06>, the membrane filtration sterility method is the method of choice for suitability of filterable pharmaceutical products. In this method, the product has been passed through a membrane filter and then <100 CFU of the QC organism is inoculated in the rinsing fluid and filtered through the same membrane.

The membrane filtration can be:

a. Open Membrane Filtration (OMF) or

b. Closed Membrane Filtration method

In both types of membrane filtration methods, the growth of the product containing media vessels will be compared with the control vessel (without the product). This is to ensure that growth of the QC organisms was not inhibited by the presence of antimicrobial substances in the product. The growth of the vessel containing product and without product must be comparable to pass the test.

Clostridium sporogenes, Pseudomonas aeruginosa, and *Staphylococcus aureus* organisms are inoculated in Fluid Thioglycollate Medium and incubated at 30 °C - 35 °C for 3 days.

Similarly, *Aspergillus brasiliensis*, *Bacillus subtilis*, and *Candida albicans* are inoculated Tryptone Soya Broth at 20 °C - 25 °C for 5 days (fungi) & 20 °C - 25 °C for 3 days (bacteria).

2. Direct inoculation

In this method, the product has been directly inoculated into suitable culture media and spiked with <100 CFU of QC organism. The product is then placed into the incubator for 3 days for bacterial growth and 5 days for fungi growth. *Clostridium sporogenes*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* organisms are inoculated in Fluid Thioglycollate Medium and incubated at 30 °C - 35 °C for 3 days.

Similarly, *Aspergillus brasiliensis*, *Bacillus subtilis*, and *Candida albicans* are inoculated Tryptone Soya Broth at 20 °C - 25 °C for 5 days (fungi) & 30 °C - 35 °C for 3 days (bacteria).

1. a. Method suitability: Open Membrane Filtration (OMF)

Step 1: Pass the liquid sample through filter membrane (with pore size not greater than 0.45 µm)

Step 2: To neutralize and remove any antimicrobial residue from the filter membrane (with pore size not greater than 0.45 µm) rinse it with 100 mL of suitable rinsing fluid

Step 3: Repeat step-2

Step 4: Add indicated test organisms < 100 CFU to the rinsing fluid and filter

Step 5: On completion of step-4, remove the filter with help of sterile forceps and cut the filter into two pieces with sterile scissors and place the same into appropriate medium

Step 5a: Add one portion of filter in Tryptone Soya Broth (CM0129B); incubate the media at 22.5 °C ± 2.5 °C for 5 days for fungi and 3 days for bacteria

Aerobic condition:
Candida albicans, Aspergillus brasiliensis; *Bacillus subtilis*

Step 5b: Add other half portion of filter in Fluid Thioglycollate Medium (CM0173B), incubate at 32.5 °C ± 2.5 °C for 3 days for bacteria

Aerobic condition:
Pseudomonas aeruginosa, Staphylococcus aureus, Clostridium sporogenes

PASSED	FAILED
Clear growth comparable to positive control	No or inhibited growth comparable to positive control, modify the test and repeat

1. b. Method suitability: Membrane Filtration through Closed Membrane Filtration

Step 1: Rinse the filter membrane (of not more than 0.45 µm size) of each canister with appropriate rinsing fluid

Step 2: On completion of step 1, immediately filter the product

Step 3: To neutralize and remove any antimicrobial residue from the filter membrane, rinse the membrane twice with 100 mL of suitable rinsing fluid

Step 4: Add indicated test organisms at < 100 CFU add to the rinsing fluid and filter through membrane filter

Step 5: On completion of step 4, add appropriate medium in the respective canister

Step 5a: Add Tryptone Soya Broth (CM0129B) to the canister and incubate it at 22.5 °C ± 2.5 °C and incubate for not more than 5 days

Aerobic condition: *Candida albicans, Aspergillus brasiliensis, Bacillus subtiis*

Step 5b: Add Fluid Thioglycollate Medium (CM0173B) to the canister and incubate it at 32.5 °C ± 2.5 °C and incubate for not more than 3 days

Aerobic condition: *Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Clostridium sporogenes*

PASSED	FAILED
Clear growth comparable to positive control	No or inhibited growth comparable to positive control; modify the test and repeat

2. Method suitability: Direct Inoculation

Step 1: Sample is inoculated in the suitable media directly with the help of syringe

Step 2: Inoculate < 100 CFU of QC organisms to the media containing sample

Step 3a: Tryptone Soya Broth (CM0129B) spiked with product and QC organism incubated at 22.5 °C ± 2.5 °C, 5 days for fungi and 3 days for bacteria

Aerobic condition: *Candida albicans, Bacillus subtilis, Aspergillus brasiliensis*

Step 3b: Fluid Thioglycollate Medium (CM0173B) spiked with product and QC organisms incubated at 32.5 °C ± 2.5 °C for 3 days

Aerobic condition: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Clostridium sporogenes*

PASSED	FAILED
Clear growth comparable to positive control	No or inhibited growth comparable to positive control; modify the test and repeat

Sterile manufacturing & regulatory

The pharma manufacturing process always keeps patient health in mind and due to this, all of the drug manufacturing processes follow stringent regulatory parameters. To ensure the regulations have been adequately followed, regulatory bodies audit the drug manufacturing sites and delve into the steps involved in:

- Raw material supply
- Data integrity compliance
- Quality management system
- Sterility procedures
- cGMP processes
- Training records of the associated employees, accreditation for the manufacturing site
- Risk management approach used during each step of drug manufacturing.

And in case the product receives any quality complaint, they utilize a system, to analyze the failure and implement corrective and preventive action to be taken for the safeguard of the product.

Sterility assay for the product

To check the sterility of the product, each raw material and component used in the drug manufacturing must pass the sterility test. For sterile products, the expectation and limit for the contamination has been already set by the regulatory bodies. Any out of specification product must be rejected an investigation must be carried out to identify the root cause.

The analysis must be carried out by using:

1. Membrane Filtration and
2. Direct inoculation

In each of the mentioned methods, the product sterility is investigated using appropriate culture media for recommended temperature and duration.

1. a. Product sterility: Open Membrane Filtration (OMF)

Step 1: Pass the liquid sample through filter membrane (with pore size not more than 0.45 µm)

Step 2: To neutralize and remove any antimicrobial residue from the filter membrane (with pore size not more than 0.45 µm) by washing it with 100 mL of suitable rinsing fluid

Step 3: Repeat step-2

Step 4: Upon completion of step 3, remove the filter with help of sterile forceps and cut the filter into two pieces with sterile seizer and place each piece in appropriate medium

Step 5a: Incubate half of the filter with Tryptone Soya Broth (CM0129B) at 22.5 °C ± 2.5 °C for 14 days

Step 5b: Incubate the other half of the filter in Fluid Thioglycollate Medium (CM0173B), at 32.5 °C ± 2.5 °C for 14 days

PASSED	FAILED
No growth	Growth, repeat

1. b. Product sterility: Membrane Filtration through Closed Membrane Filtration

Step 1: Rinse the filter membrane (of not more than 0.45 µm size) of each canister with appropriate rinsing fluid

Step 2: On completion of step-1, immediately filter the product

Step 3: To neutralize and remove any antimicrobial residue from the filter membrane, rinse the membrane twice with 100 mL of suitable rinsing fluid

Step 4: On completion of step 3, add appropriate medium in the respective canister

Step 5a: Add Tryptone Soya Broth (CM0129B) to the canister and incubate it at 22.5 °C ± 2.5 °C for 14 days

Step 5b: Add Fluid Thioglycollate Medium (CM0173B) to the canister and incubate it at 32.5 °C ± 2.5 °C for 14 days

PASSED	FAILED
No growth	Growth, repeat

2. Product sterility: Direct Inoculation

Step 1: Sample were spiked in the suitable media directly with the help of syringe

Step 2a: Tryptone Soya Broth (CM0129B) spiked with product incubated at 22.5 °C ± 2.5 °C for 14 days

Step 2b: Fluid Thioglycollate Medium (CM0173B) spiked with product incubated at 32.5 °C ± 2.5 °C for 14 days

PASSED	FAILED
No growth	Growth, repeat

Media fills or process simulation

To ensure the aseptic conditions of the drug manufacturing, media fill (MFs) are performed. In this process, the microbial culture media has been prepared and passed through the similar condition of the drug manufacturing and then media containing vials must be incubated to observe whether there is any microbial growth. To observe the same the media were passed through the various steps of drug manufacturing and then kept for a period of total 14 days, 7 days each at 20 °C - 25 °C & 30 °C - 35 °C, respectively.

Media Fills are designed to evaluate:

- Aseptic assembly
- Operation of critical (sterile) equipment
- Qualifying operators skills and techniques
- Demonstrate the environmental conditions of the sterile drug manufacturing.

Media fills do not validate the ability of a filter to sterilize the microbial culture media.

Regulatory requirements

- Media fills or process validation are a requirement of current Good Manufacturing Practices (cGMP) for finishes pharmaceuticals (21 CFR 211) and GMP regulations for medical devices (21CFR 820) and, therefore, these are applied for manufacture for both drug products and medical devices
- <71> Sterility
- <797> Pharmaceutical Compounding – Sterile Preparations
- <1116> Microbiological Control and Monitoring of Aseptic Processing Environments
- ISO 13408-1:2008

FDA recommendation for key factors in MF program

- Aseptic assembly of equipment (e.g., at start-up, during processing)
- Longest batch run must be considered for risk assessment
- Number of personnel and their activities
- All the shift changes, breaks, and gown changes must be included sanitization activities
- Aseptic sample collections
- Line speed and configuration
- Fill volume and weight checks
- Container closure systems

Fig: cfTSB (DCM) & Fig: cfTSB (in biopressing container)

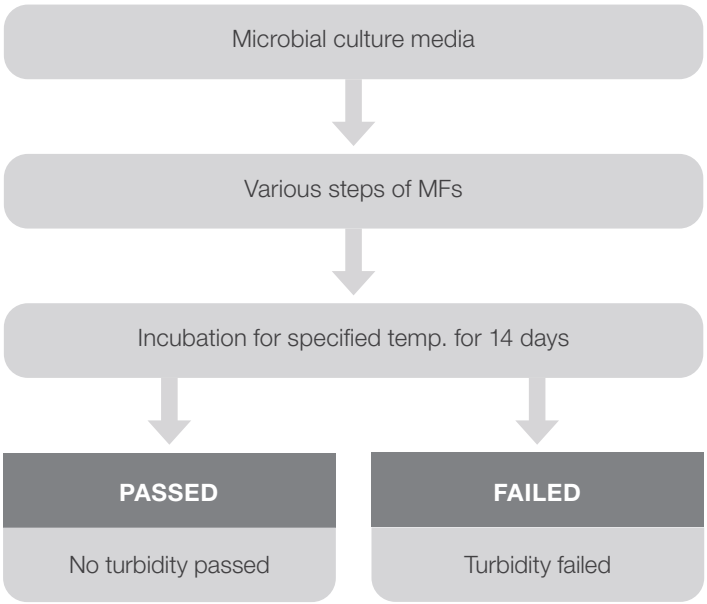
Media fill process

1. **Preparation of growth media:** Preparation of the growth media is the first step in the media fills. In most cases, this media will be **Tryptone Soya Broth (TSB)**, alternatively known as **Soybean Casein Digest Medium (SCDM)**, which are mostly performed under aerobic condition (in presence of oxygen). Although there are certain conditions where media fill trials require anaerobic condition (absence of oxygen and presence of carbon dioxide), sometimes Alternate Fluid Thioglycollate Broth (the media exclude resazurin dye and agar) will be used to validate the sterile manufacturing for those applications. For some other applications, Mannitol Broth, Tryptose Phosphate Broth, Lactose Broth or Peptone Broth, etc. were also used in media fills activities.
   - Growth promotion studies must be carried out with environmental isolates apart from standard growth promotion QC organisms.
   - To avoid Mycoplasma contamination from TSB media, the drug manufacturing organizations prefer to buy a sterile TSB product.
   - Further, most of the TSB media contains fibers, which can result in the filter clogging, resulting in the repetition of the complete process and investigation of the root cause. For confidence in filtration, Oxoid Cold Filterable TSB has been designed with highly purified peptones. In addition, we also provide the filterability for each media lot in terms of V_{max}, which are validated on three different types of filter membranes, i.e., PVDF, Nylon and PES.
2. **Line clearance:** Prior to the use, all the equipment and ancillary must be sterilized or sanitized.
3. **Operators:** Operator performs the media fill processes, as close as possible to the original process, including the aseptic techniques, routine manipulations, number of personnel, line speeds, length of run etc.
4. **Environmental monitoring program data**
   The environmental monitoring data has been captured for the entire media fill activities and reviewed to understand the EM trend during the said period.
5. **Incubation:** The media-containing vials, ampoules, syringes, etc. or bulk media are collected and incubated for 14 days at an appropriate temperature (typically 7 days at 20 °C - 25 °C and 7 days at 30 °C – 35 °C) or as per set SOP of organization
6. **Interpretation:**
   - If there is no growth, the media are tested for growth promotion using standard test organisms plus routine environmental organisms. On successful growth promotion, the aseptic process simulation can be considered successful.
   - In case the number of vials are within alert limit the media fills are still considered successful.
   - If it has progressed to action level, then re-investigation needs to be carried out and must be correlated with the previous media fills results.

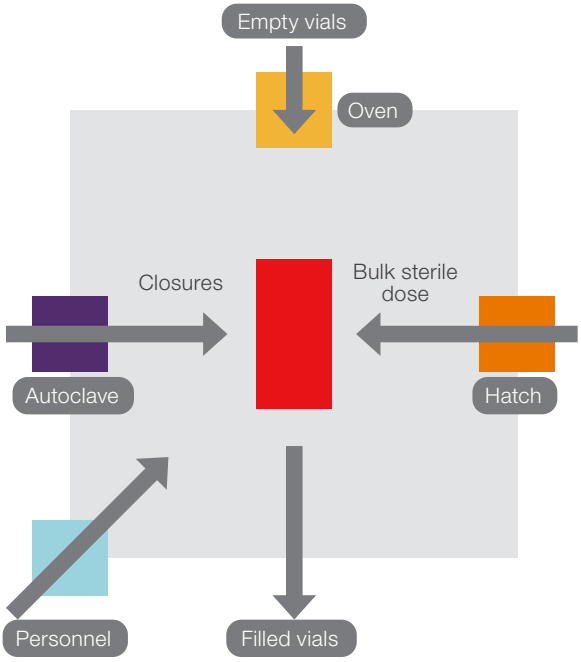
Media fill can be divided into types:

1. **Solid media fills**
   Some media fills require solid filling during the initial stage, thus for this kind of media trial, dehydrated culture media must be utilized.
2. **Liquid media fills**
   Some media fills require liquid filling, thus for this kind of media trial, media in BioProcess Containers must be utilized, although some drug manufacturers still request dehydrated culture media for this application.

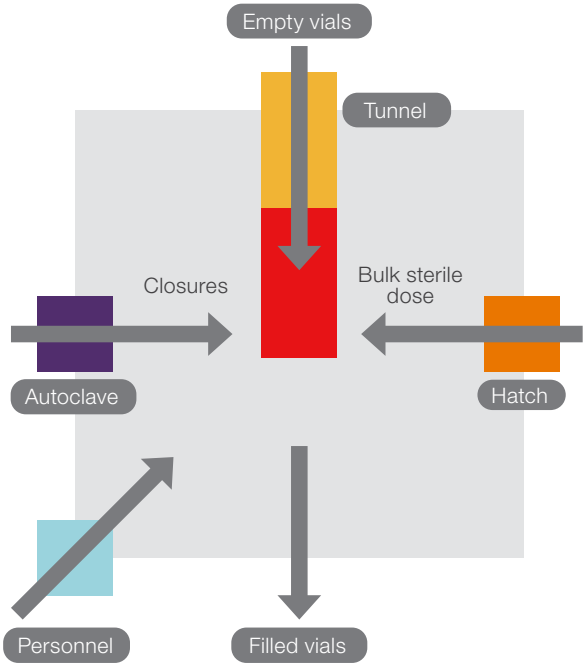
Schematic representation for media fill result interpretation



Schematic representation of solid media fill specimen

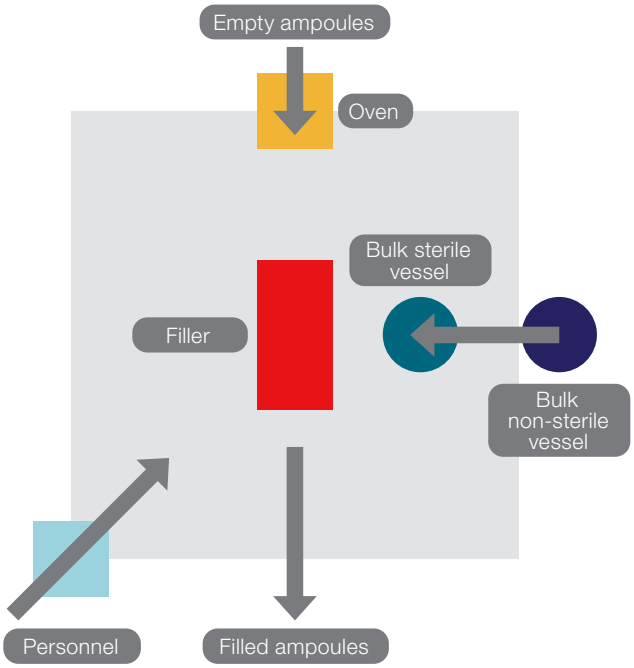


Vials are depyrogenated in a double door oven and then manually loaded into the filling machine

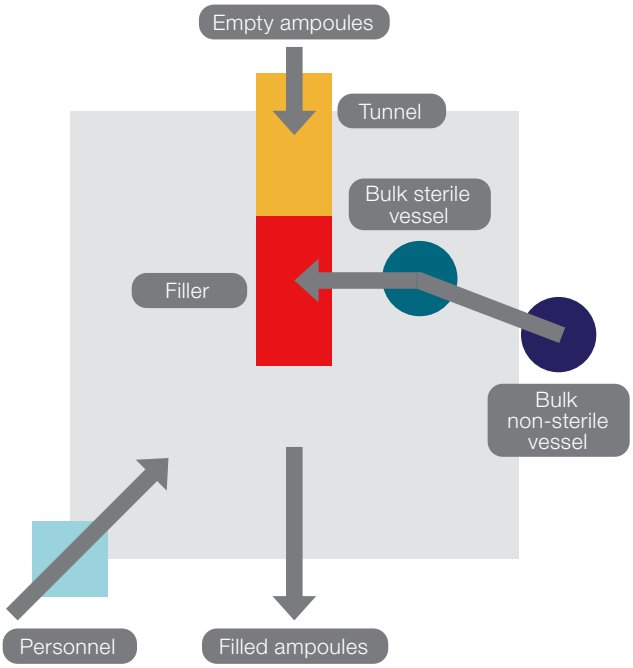


Vials are depyrogenated in a tunnel which is linked to the filling machine

Schematic representation of liquid media fill specimen



Ampoules are depyrogenated in a double door oven and then manually loaded into the filling machine



Ampoules are depyrogenated in a tunnel which is linked to the filling machine

Key

Filling room | Support clean area

Sterility testing:

Sterility media and media for process simulation

Sampling and growth

Product	Format	Product code
Fluid Thioglycollate Medium	100 mL bottle w/septum	R112646
	500 mL bottle w/septum	R112642
	100 mL serum bottle	R112641
	100 mL wide mouth bottle	R112640
	100 mL bottle w/septum dbl bagged	R112976
	100 mL serum bottle dbl bagged	R112647
	100 mL WMB dbl bagged	R112997
	100 mL screw cap bottle	R112910
	100 mL septum	BO0368M
	500 mL WMB w/septum	BO0510V
	100 mL WMB w/septum	BO0510M
	100 mL sirop (serum) screw cap bottle	BO0211M
Fluid Thioglycollate Medium, Dehydrated	500 g	R453452
	2.5 kg	R453454
Tryptic Soy Broth (TSB)Δ	10 mL tube, 20 /pk	R117834
	100 mL bottle w/septum	R112745
	500 mL bottle w/septum	R112732
	1000 mL bottle	R112740
	100 mL serum bottle	R112731
	100 mL wide mouth bottle	R112730
	10 mL vial w/septum	BO0369E
	500 mL WMB w/septum	BO0509V
	100 mL WMB w/septum	BO0509M
	100 mL vial w/septum	BO0369M
	90 mL sirop(serum) screw cap bottle	BO0351U
	100 mL sirop(serum) screw cap bottle	BO0351M
	100 mL screw bottle dbl bagged	R112912
	100 mL septum bottle dbl bagged	R112986
	100 mL serum bottle dbl bagged	R112751
	100 mL WMB dbl bagged	R112996
Vegetable Peptone Broth (VPB)	500 g	VG0101B
	5 kg	VG0101T
Cold Filterable TSB	500 g	CM1065B
	5 kg	CM1065T
Cold Filterable Vegetable Peptone Broth	500 g	VG0104B
	5 kg	VG0104T

Δ Tryptic Soy Broth (TSB) = Soybean-Casein Digest Broth * alternate to FTM

Sterile media fills

Product	Format	Product code
Cold Filterable TSB	20 L	BP1065E
	1 L	BP1065A
Cold Filterable Vegetable Peptone Broth	20 L	BP0104E
	1 L	BP0104A
Cold Filterable Tryptone Soya Broth	10 L	BP1065C
Cold Filterable Veg. Peptone Broth	10 L	BP0104C

Rinsing and diluting fluids

Product	Format	Product code
Fluid A	300 mL bottle w/septum	R112312
	1000 mL polypropylene bottle	R112314
	100 mL serum bottle	R112490
	300 mL serum bottle	R112311
	100 mL Din bottle	BO0833M
	300 mL Din bottle	BO0833Z
	500 mL Din bottle	BO0833V
	300 mL sirop	BO0619X
Fluid D	100 mL bottle	R112323
	300 mL bottle w/septum	R112322
	300 mL serum bottle	R112321
	100 mL wide mouth bottle	R112325
	310 mL Din bottle	BO0964Z
Fluid K	Fluid K 100mL serum bottle	R112332

Environmental monitoring

Category	Product	Format	Product code
Dehydrated culture media	D/E Neut Broth	500 g	R453042
	D/E Neut Agar	500 g	R453032
Contact plates	Sterile D/E Neutralizing Agar	10 /pk, Double + bag	R111803
	Sterile Sabouraud Dextrose Agar w/ Lecithin	10 /pk, Double + bag	R111805
	Sterile Tryptic Soy Agar w/Lecithin, Polysorbate 80	10 /pk, Double + bag	R111800
Triple wrap contact plates	Triple Wrap Sterile Tryptone Soya Agar with neutralizers	100 /pk	PO5511D
Settling plates	Sterile Tryptic Soy Agar	10 /pk, Double + bag	R111870
Triple wrap settling plates	Triple Wrap Sterile Tryptone Soya Agar	100 /pk	PO5500B R111816TW
	Triple Wrap Sterile Tryptone Soya Agar with neutralizers	100 /pk	PO5501B PO5501B
Storage bag for triple wrap settling plate	Cellophane Bags	10 p/k	R111551
Swabs	Sani-Cult™	5 mL, 100 /pk	R723141

Mycoplasma transport and isolation

Product	Format	Product code
Thermo Scientific™ MicroTest M4™	3 mL tube, 12 /pk	R12502
	3 mL tube, 72 /pk	R12500
Thermo Scientific™ MicroTest M5™	3 mL tube, 12 /pk	R12516
	3 mL tube, 72 /pk	R12515
Thermo Scientific™ MicroTest M6™	1.5 mL tube, 12 /pk	R12535
	1.5 mL tube, 72 /pk	R12530
10B Arginine Broth	1.8 mL tube (15 x 45 mm)	R20305
A-8 Agar	10 /pk monoplate	R20205
A-8 Agar, selective	10 /pk monoplate	R20204
SP4 Glucose Agar/Broth	10 /pk monoplate	R20276
	100 mL clear square bottle	R112585
PPLO Agar/Broth	10 /pk monoplate	R20261
	15 x 60 mm plate	R20260
	5 mL tube	R20360
Mycoplasma Broth Base	500 g	R454172
Mycoplasma Broth, Frey	500 g	R454162

Quality control organisms

Product	Format	Product code
Aspergillus brasiliensis ATCC® 16404™	Thermo Scientific™ Quanti-Cult™: 10 tests/kit	R4731100
	Thermo Scientific™ Quanti-Cult Plus™: 100 tests/kit	R4711100
	Thermo Scientific™ Culti-Loops: 5 Loops/pack	R4601100
Bacillus subtilis ATCC® 6633™	Thermo Scientific™ Quanti-Cult™: 10 tests/kit	R4731221
	Thermo Scientific™ Quanti-Cult Plus™: 100 tests/kit	R4711221
	Thermo Scientific™ Culti-Loops: 5 Loops/pack	R4601221
Burkholderia cenocepacia ATCC® BAA-245™	Thermo Scientific™ Quanti-Cult Plus™: 100 tests/kit	R4715221
Burkholderia cepacia ATCC® 25416™	Thermo Scientific™ Quanti-Cult Plus™: 100 tests/kit	R4715220
	Thermo Scientific™ Culti-Loops: 5 Loops/pack	R4605220
Burkholderia multivorans ATCC® BAA-247™	Thermo Scientific™ Quanti-Cult Plus™: 100 tests/kit	R4715222
Candida albicans ATCC® 10231™	Thermo Scientific™ Quanti-Cult™: 10 tests/kit	R4731503
	Thermo Scientific™ Quanti-Cult Plus™: 100 tests/kit	R4711503
	Thermo Scientific™ Culti-Loops: 5 Loops/pack	R4601503

Quality control organisms (continued)

Product	Format	Product code
Clostridium sporogenes ATCC® 11437™	Thermo Scientific™ Quanti-Cult™: 10 tests/kit	R4731703
	Thermo Scientific™ Quanti-Cult Plus™: 100 tests/kit	R4711703
	Thermo Scientific™ Culti-Loops™: 5 Loops/pack	R4601703
Clostridium sporogenes ATCC® 19404™	Thermo Scientific Quanti-Cult Plus: 100 tests/kit	R4711700
	Thermo Scientific Culti-Loops: 5 Loops/pack	R4601700
Escherichia coli ATCC® 8739™	Thermo Scientific Quanti-Cult: 10 tests/kit	R4737085
	Thermo Scientific Quanti-Cult Plus: 100 tests/kit	R4717085
	Thermo Scientific Culti-Loops: 5 Loops/pack	R4607085
Kocuria rhizophila ATCC® 9341™	Thermo Scientific Quanti-Cult Plus: 100 tests/kit	R4714075
	Thermo Scientific Culti-Loops: 5 Loops/pack	R4604075
Pseudomonas aeruginosa ATCC® 9027™	Thermo Scientific Quanti-Cult: 10 tests/kit	R4735210
	Thermo Scientific Quanti-Cult Plus: 100 tests/kit	R4715210
	Thermo Scientific Culti-Loops: 5 Loops/pack	R4605210
Salmonella enterica subsp. *enterica* serovar Typhimurium ATCC® 14028™	Thermo Scientific Quanti-Cult Plus: 100 tests/kit	R4716000
	Thermo Scientific Culti-Loops: 5 Loops/pack	R4606000
Salmonella sp. serovar Abony NCTC 6017	Thermo Scientific Quanti-Cult Plus: 100 tests/kit	R4716007
	Thermo Scientific Culti-Loops: 5 Loops/pack	R4606007
Staphylococcus aureus ATCC® 6538™	Thermo Scientific Quanti-Cult: 10 tests/kit	R4737016
	Thermo Scientific Quanti-Cult Plus: 100 tests/kit	R4717016
	Thermo Scientific Culti-Loops: 5 Loops/pack	R4607016

Environmental monitoring

Category	Product	Format	Product code
Thermo Scientific™ Isolator Wrap™ Sterile Contact Plates	Sterile D/E Neutralizing Agar	100 /pk, Foil barrier wrap + bag	R111824
	Sterile Sabouraud Dextrose Agar w/ Lecithin, Polysorbate 80	10 /pk, Foil barrier wrap + bag	R111825
	Sterile Tryptic Soy Agar w/Lecithin, Polysorbate 80	10 /pk, Foil barrier wrap + bag	R111820

Environmental monitoring program

The environmental monitoring (EM) program is an important tool to provide vital information regarding environmental condition of cleanrooms, which includes isolators, RABS (Restricted Access Barrier System), manufacturing area and surroundings, personal hygiene and equipment sterility conditions pre- and post-operational conditions.

EM program are key indicators, which help the manufacturer to understand whether their operational and non-operational conditions in cleanroom areas are complying to the stringent regulatory requirement.

Cleanroom requirement in pharma

The cleanroom set up is required to maintain the sterile environment and cleanliness in and around the manufacturing regions. Regulatory bodies like the FDA emphasize the need for implementation of cleanroom set-up in pharma industries. Cleanrooms utilize HVAC (Heating, Ventilating and Air Conditioning) systems which contain HEPA filters to remove particles.

Types of environmental monitoring program

1. Air monitoring
   a. Active air monitoring
   b. Passive air monitoring
2. Surface monitoring
3. Personal monitoring

Cleanroom area and surrounding

Common cleanroom classification system

No.	FDA grade system	ISO grade system	FED STD 209E
1	A	ISO 5	Class 100
2	B	ISO 6	Class 1000
3	C	ISO 7	Class 10000
4	D	ISO 8	Class 100000

Please note: That all mentioned classifications are equivalent and used as synonyms to one another.

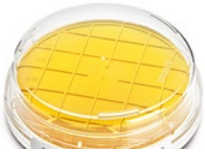
1. a. Active air monitoring

- Regulatory requirements- ISO 14644, Fed Std-209E, USP <1116>
- Quantitative and semi-quantitative method monitoring
- Air sampler utilizes Tryptone Soya Agar and Tryptone Soya Agar with neutralizers media plates for active air monitoring
- Ideally 100 Liters/Min of air has been forcefully introduced to the plates through air sampler for 10 mins
- After air sampling the media plates were incubated for defined period and results were then studied

Air Sampler

90mm Diameter Settle Plate

55mm Diameter Contact Plate



Step 1: Affix Media plate in Air Sampler

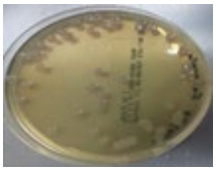
Step 2: Setting the air sampling volume and time

Step 3: After completion of air sampling close the lid & place the media plate in incubator

Step 4: Plate inspection

Negative growth

Positive growth



1.b. Passive air sampling or settling plates method

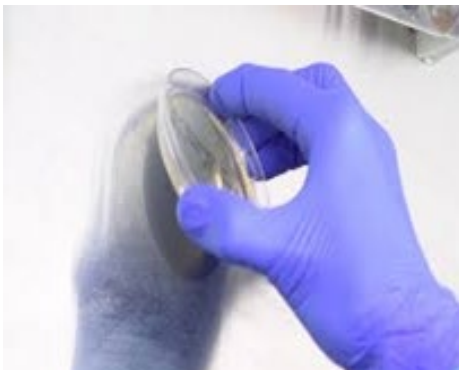
- Regulatory- ISO 14644, FDA Standard-209E, USP <1116>; USP <797>
- In this method, 90mm or settle plate will be exposed for 4 hours, so that for the said duration the particles can settle down to the plate
- Qualitative or semi-quantitative method
- Values are for guidance only - not intended to represent specifications
- Ideally a medium, which can promote growth for both has to be considered for the application, e.g., SCDA or TSA plates, or in some injectable and beta-lactamase plants, it is required that these TSA media plates must contain neutralizers
- Areas where there is little air movement (i.e. “dead spaces”) or where airflows converge or are excessively turbulent

Settle plate

2. Surface/personal monitoring

Contact plate use in surface monitoring

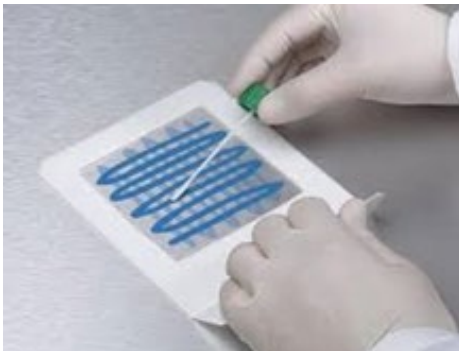
- Regulatorys- ISO 14644, Fed Std-209E, USP <1116>
- Product contact surfaces, floors, walls, and equipment should be tested through contact plates
- Contact plates - used for flat surfaces, personnel monitoring sample area of 25 cm^2
- Ideally a medium which can promote growth for both has to be considered for the application
- All the plates used for the contact purposes must contain neutralizers to ensure the number of microorganisms per area sampled
- The grid on contact plates allows counting of CFU per cm^2



Contact plate

Sterile swab use in surface monitoring

- These are used in surface monitoring of irregular and difficult to reach surfaces
- Like contact plate, surface swabs sampling also are also performed for 25 cm^2 area
- Qualitative or quantitative
- Surface monitoring should be performed to minimize risk of contaminating critical surfaces during production in aseptic processing



Swab sampling

3. Personal monitoring

Fingertip test in personal monitoring

- Both settle (90 mm) plates and contact (55 mm) plates are used for “finger dab” or more commonly known as the fingertip test for personal monitoring (Fig-17)
- In this test the operator must touch the media plate with their gloves- pre- and post-manufacturing activities, to ensure that they are not carrying any contamination, or that the sanitation process is adequate to control contamination
- Positive samples need to be reviewed to provide information on whether product complies to the set acceptance limit

“Fingertip” test

Gown sampling in personal monitoring

- In this test the operator gowning process must undergo testing with contact plates- pre- and post-manufacturing activities, to ensure that they are not carrying any contamination, or that the gowning process is adequate to control contamination
- Positive results need to be reviewed, to verify whether or not they are within the acceptable limit

Gown sampling

Room classification	Active air sample (%)	Settle plate (90 mm) 4th exposure (%)	Contact plate or swab (%)	Glove or garment (%)
Isolator/closed RABS (ISO 5 or better)	< 0.1	< 0.1	< 0.1	< 0.1
ISO 5	< 1	< 1	< 1	< 1
ISO 6	< 3	< 3	< 3	< 3
ISO 7	< 5	< 5	< 5	< 5
ISO 8	< 10	< 10	< 10	< 10

Classification	In operations (dynamic) routine particulate sampling
Grade A (filling operation)	For the full duration of operation
Grade B	Daily[1]
Grade C	Weekly
Grade D	Not required
UDAF work stations in B	Daily[1]
UDAF work stations in C	Weekly
UDAF work stations in D	Monthly
UDAF in UNC areas	Routine re-qualification of UDAF is sufficient

Non-sterile pharmaceutical industry

Unlike sterile pharma manufacturing, the manufacturing of non-sterile pharma products does not require the cleanroom environment to be completely free from any microbial presence.

There are acceptance limits set for these industries, which align with the regulatory acceptance guidelines to manufacture non-sterile products. Further, they test for objectionable microorganism presence in the product.

Microbial identification of non-sterile products

Two basic tests done in non-sterile pharmaceutical industry for microbial identification. They are as follow:

1. Microbial enumeration test
2. Absence of microorganisms in raw material and finished goods

There are four major chapters in the USP (US Pharmacopeia), which provide information on the above mentioned.

1. USP<60> Microbial Examination of Non-Sterile Products: Tests for Burkholderia Cepacia Complex
2. USP<61> Microbial Examination of Non-Sterile Products: Microbial Enumeration Test
3. USP<62> Microbial Examination of Non-Sterile Products: Test for Specified Microorganism
4. USP <1111> Microbial Examination of Non-Sterile Products: Acceptance Criteria for Pharmaceutical Preparation and Substance for Pharmaceutical use

Growth promotion, indicative and inhibitory properties of the dehydrated (microbial) culture media

As mentioned in the USP <60>, <61>, and <62>, prior to the use of all the dehydrated (microbial) culture media or prepared media, must be challenged with microorganism strains (QC organisms) for growth promotion, indicative and inhibitory properties as applicable.

Handling note for the microbial strains (QC organisms)

- Culture must be stored as per manufacturer instructions, e.g.: Thermo Scientific™ Quanti-Cult Plus™ must be stored 2 °C - 8 °C.
- The culture strains should not be used beyond 5th (Fifth) Passage or subculture
- Inoculate not more than 100 or equal to 100 for growth promotion, not less than 100 for inhibitory properties

Based on the non-sterile requirement, the QC organisms are challenged in Tryptone Soya Broth/ Tryptone Soya Agar for bacterial growth at 30 °C - 35 °C for ≤ 3 days and Potato Dextrose Agar for yeast and mold growth 20 °C - 25 °C for ≤ 5 days. For solid media, the growth of the microbial strains (QC organisms) should not not deviate greater than a factor of 2, from a previously approved batch of the medium. For liquid media, clearly visible growth of microbial strains must occur and be comparable to a previously approved batch of the medium.

QC Organisms:

Quanti-Cult Plus workflow for liquid media broth

Quanti-Cult Plus

- Blue vial contains 1.2 mL rehydrating fluid
- Red vials contain proprietary gel preserved cultures

Proprietary-gel-preserved cultures

Spike in media bottle

Storage at 2 ˚C to 8 ˚C

Take the cultures out of refridgerator

Step 1: Take both the culture and rehydrating fluid out of the refrigerator so that it can attain room temperature

Step 2: Under bio-safety cabinet, transfer the red cap to rehydrating fluid vial and discard the blue cap

Invert the tube to rehydrate

Step 3: Incubation at 35 ˚C to 37 ˚C for 15 mins in incubator

Step 4: Mix suspension to aid dissolution

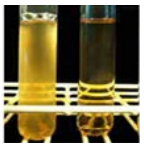
Step 5: Take 100 µL inoculum to deliver <100 CFU

Incubate at 30 °C - 35 °C (bacteria) or at 20 °C - 25 °C (fungi)

Refrigerator:

- 2 ˚C - 8 ˚C

Under biosafety cabinet change the caps



QC Organisms:

Quanti-Cult Plus Workflow for solid media

Quanti-Cult Plus
- Blue vial contains 1.2 mL rehydrating fluid
- Red vials contain proprietary gel preserved cultures

Proprietary-gel-preserved cultures

Storage at 2 ˚C to 8 ˚C

Take the cultures out of refrigerator

Step 1: Take both the culture and rehydrating fluid out of the refrigerator so that it can attain room temperature

Step 2: Under bio-safety cabinet, transfer the red cap to rehydrating fluid vial and discard the blue cap

Invert the tube to rehydrate

Step 3: Incubation at 35 ˚C to 37 ˚C for 15 mins in incubator

Step 4: Mix suspension to aid dissolution

Step 5: Take 100 µL inoculum to deliver <100 CFU

Incubate at 30 °C - 35 °C (bacteria) or at 20 °C - 25 °C (fungi)

Plating on media plate

Refrigerator:
- 2 ˚C - 8 ˚C

Under biosafety, cabinet change the caps

Method suitability testing

In this method of suitability testing, microbial culture media must be spiked with a suitable amount of the sample in conjunction with the ≤100CFU of the challenged organism (QC organisms), then these vials have to be incubated at the desired temperature for the period mentioned as per the SOP (Standard Operating Procedure). Then, growth of these organism must be compared with the positive control, which contains media and the challenge organism (≤100CFU). If the growth is comparable to the control the test, it is considered to have passed, whereas there is inhibition or no growth then the sample has to be repeated with the addition of neutralizer to inhibit the antimicrobial activity of the drug.

Method suitability must be carried out into fhe ollowing stages:

1. Product development stage- media spiked with the product is tested in conjunction with the challenging organism
2. Post manufacturing of finished goods (preferably three lots) must be tested without the challenging organism in the media. The testing must be carried out in accordance with the USP <1227>

Testing of the finished product (as per USP <62)

1. Test for absence and quantitative test- bile tolerant bacteria
2. Test for absence- *Escherichia coli*
3. Test for absence- *Salmonella*
4. Test for absence- *Pseudomonas aeruginosa*
5. Test for absence- *Staphylococcus aureus*
6. Test for absence- *Clostridia*
7. Test for absence- *Candida albicans*

Testing of the finished product (as per USP <60)

8. Test for absence- *Burkholderia cepacia* complex

Bile tolerant Gram-negative bacteria

These organisms are usually associated with an aqueous environment and indicator of poor hygiene or poor water quality. These organisms are of concern as many species are opportunistic pathogens or may cause spoilage of the product.

Enterobacter Enrichment (EE) Mossel Broth

This media has been developed by modification in the formulation of Brilliant Green Bile Broth, which contains purified ox bile (Oxgall), in place of bile salts, and Di-Sodium Phosphate to improve the buffering capacity of the medium and encourage early growth of indicator organism. Mossel EE Broth is recommended by US pharmacopeia (USP) to test for the presence of bile tolerant Gram-negative bacteria by the American Public Health Association as an enrichment broth in strict enrichment procedures. Mossel EE Broth is formulated in conformance with harmonized United States pharmacopeia and European pharmacopeia guidelines.

Mode of action

Peptones provide nitrogen carbon compounds essential for bacterial growth. Dextrose is an energy source. Di-Sodium Phosphate and Potassium are buffering agent. Brilliant Green and Oxgall are selective agents, which inhibit gram-positive bacteria.

Components	Gram/Litre
Oxgall	20.0
Peptone	10.0
Disodium phosphate	8.0
Dextrose	5.0
Monopotassium phosphate	2.0
Brilliant green	0.015

Violet-Red Bile Glucose Agar (VRBGA)

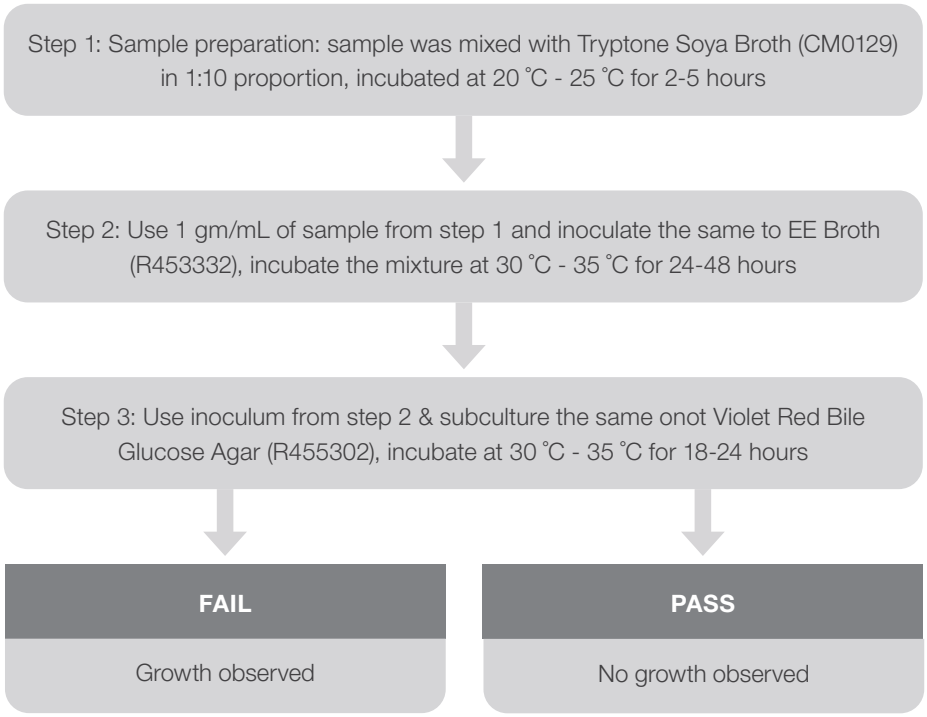
Mossel et. al. modified VRBGA by adding glucose to enable detection of non-lactose fermenting Enterobacteriaceae. Further research demonstrated that lactose could be omitted, resulting in the formulation known as Violet Red Bile Glucose Agar (VRBGA). This formulation complies to US pharmacopeia (USP) and European pharmacopeia (EP).

Mode of action

Peptones supply amino acids, peptides and nitrogenous compounds, which are essential for bacterial growth. Yeast extract provides essential B complex vitamins and glucose is the carbon energy source, which would stop sodium chloride activity and maintains osmotic equilibrium. Bile salts and crystal violets are selective agents which inhibit the growth of gram-positive organisms. Neutron red is an indicator of acid production; Gram-negative organisms which ferment glucose from colonies are pink to red in color.

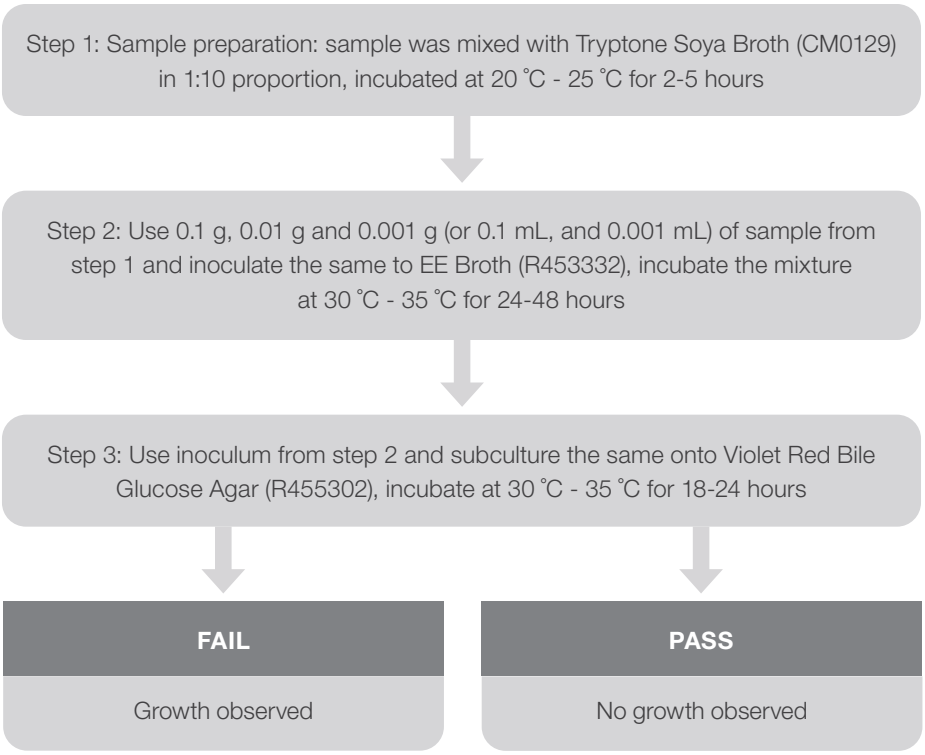
Components	Gram/Litre
Glucose	10.0
Gelatin peptone	7.0
Sodium chloride	5.0
Yeast extract	3.0
Bile salts	1.5
Neutral red	0.030
Crystal violet	0.002
Agar	15.0

Test for absence of bile tolerant Gram-negative bacteria



Product	Property	Format	Product code
Test for bile-tolerant Gram-negative bacteria			
Mossel Enterobacter Enrichment Broth	Nutritive for *E. coli* & *P. aeruginosa*; selective for *S. aureus*	500 g	R453332
Violet Red Bile Glucose Agar	Nutritive & indicative for *E. coli* & *P. aeruginosa*	15x100 mm Plate	R110097
		500g	R455302
Escherichia coli ATCC® 8739™	Growth promoting of Mossel EE broth Growth promoting & indicative of VRBG Agar	Quanti-Cult: 10 tests/kit	R4737085
		Quanti-Cult Plus: 100 tests/kit	R4717085
		Culti-Loops: 5 Loops/pack	R4607085
Pseudomonas aeruginosa ATCC® 9027™	Growth promoting of Mossel EE broth Growth promoting & indicative of VRBG Agar	Quanti-Cult: 10 tests/kit	R4735210
		Quanti-Cult Plus: 100 tests/kit	R4715210
		Culti-Loops: 5 Loops/pack	R4605210
Staphylococcus aureus ATCC® 6538™	Inhibitory of Mossel EE broth	Quanti-Cult: 10 tests/kit	R4737016
		Quanti-Cult Plus: 100 tests/kit	R4717016
		Culti-Loops: 5 Loops/pack	R4607016

Quantitative test for bile tolerant Gram-negative bacteria



Please note that the result interpretation is determined by the table below:

Results for each quantity of product			Probable number of bacteria per g or mL of product
0.1 g or 0.1 mL	0.01 g or 0.01 mL	0.001 g or 0.001 mL	
+	+	+	more than 10^3
+	+	–	less than 10^3 and more than 10^2
+	–	–	less than 10^2 and more than 10
–	–	–	less than 10

Escherichia coli

This is a Gram-negative bacterium and is an indicator for faecal contamination. Such contamination could arise from poor hygiene of operators, contamination from animals, cats, birds or low-quality water supply among guest others. *Escherichia coli* can cause diarrhea and sickness, and some strains can produce potent verotoxins.

MacConkey Broth

MacConkey Broth Is a modification of the original bile salt broth first described by MacConkey in 1900, which contained litmus as an indicator and sodium taurocholate to inhibit Gram positive organisms. Oxgall inhibits growth of Gram-positive organisms and replaces sodium taurocholate used in the original formulation. The formulation for MacConkey Broth is in conformance with Harmonized Pharmacopeia (United States Pharmacopeia and European Pharmacopoeia guidelines).

Mode of action

Peptone provides nitrogenous compounds and amino acids necessary for bacterial growth. Lactose serves as a carbon source. The selective agent Oxgall inhibits the growth of most Gram-positive organisms. Lactose and bromocresol indicator enable the differentiation of lactose fermenting Gram-negative bacilli. Lactose fermenters (i.e. coliforms) cause the medium to be changed from purple to yellow and produce gas bubbles.

Components	Gram/Litre
Gelatin peptone	20.0
Oxgall	5.0
Lactose	10.0
Bromocresol purple	0.01

MacConkey Agar

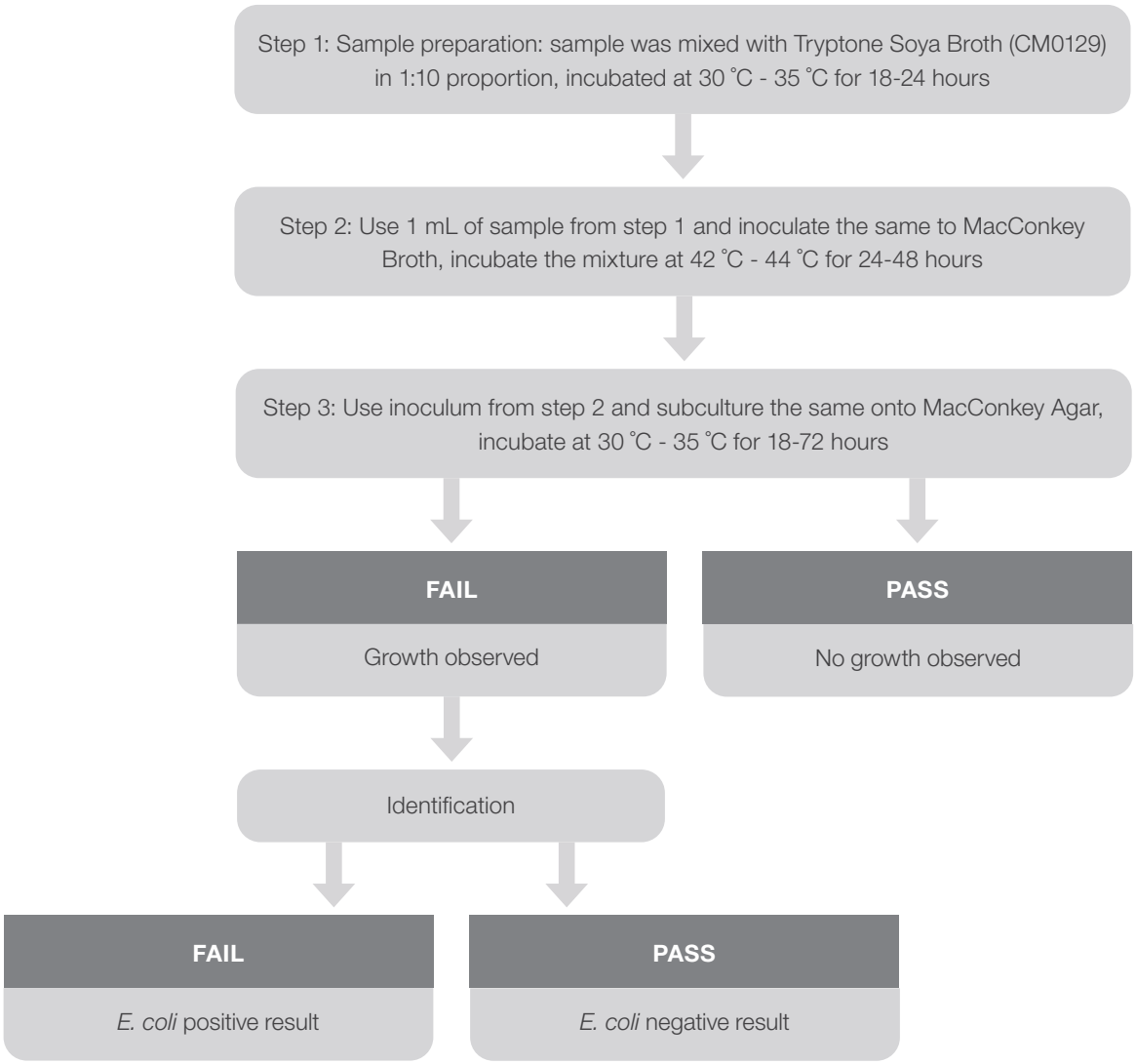
In 1900, MacConkey utilized Neutral Red Bile Salt Medium for cultivation and identification of enteric organisms. A detailed description of selective and a differential property of the medium was published in 1905. Over the years, MacConkey's original formula was modified; the agar content has been reduced, the concentration of bile salts and neutral red has been adjusted and sodium chloride has been added. The modification of MacConkey Agar which results has demonstrated improved inhibition of swarming by *Proteus* species.

Mode of action

In this formulation, peptones provide nitrogenous nutrients and amino acids necessary for growth. Lactose serves as a carbon source. Sodium chloride supplies essential electrolytes and maintains osmotic equilibrium. The crystal violet and bile salts are the selective agents which inhibit growth of most of Gram-positive organisms. Differentiation of Gram-negative bacilli is accomplished through the addition of lactose and neutral red, a color indicator.

Components	Gram/Litre
Gelatin peptone	17.0
Meat peptone	1.5
Lactose	10.0
Neutral red	30.00
Sodium chloride	5.0
Crystal violet	1.0
Bile salts	1.5
Casein peptone	1.5
Agar	13.5

Test for absence of *Escherichia coli*



Product	Property	Format	Product code
Test for *Escherichia coli*			
MacConkey Broth	Nutritive for *E. coli* & selective for *S. aureus*	15 x 103 mm tube, 5 mL	R061336
		500 g	R453822
MacConkey Agar	Nutritive & indicative for *E. coli*	Monoplate	R01550
		500 g	R453802
Escherichia coli ATCC® 8739™	Growth promoting of MacConkey Broth Growth promoting & indicative of MacConkey Agar	Quanti-Cult: 10 tests/kit	R4737085
		Quanti-Cult Plus: 100 tests/kit	R4717085
		Culti-Loops: 5 Loops/pack	R4607085
Staphylococcus aureus ATCC® 6538™	Inhibitory of MacConkey Broth	Quanti-Cult: 10 tests/kit	R4737016
		Quanti-Cult Plus: 100 tests/kit	R4717016
		Culti-Loops: 5 Loops/pack	R4607016

Salmonella

Salmonella enterica serovar Typhimurium is a microbe of fecal origin, which can cause severe diarrhea and sickness. The resultant dehydration in humans can be potentially fatal for children and elderly age groups due to their compromised immunity.

Rappaport Vassiliadis Salmonella Enrichment (RVS) Broth (USP/EP/JP)

Rappaport et.al. formulated an enrichment medium for the selective recovery of salmonella species. Vassiliadis et. al. modified the formulation by reducing the concentration for malachite green and magnesium chloride creating Rappaport Vassiliadis (RV) Broth. RVS Broth has been formulated as per Harmonized Pharmacopeia.

Mode of action

Soya peptone is the source of carbon and nitrogen. Magnesium chloride maintains the osmotic pressure and potassium dihydrogen phosphate acts as buffer. Malachite green and magnesium chloride help the medium provide a selective environment, which helps in the recovery of *Salmonella* spp. from contamination sources.

XLD Agar

The medium was developed by Taylor for selective isolation and differentiation of enteric pathogens, especially *Shigella*. Since the formulation was designed, the XLD Agar has been satisfactorily delivering the recovery for *Salmonella* spp. in various industries.

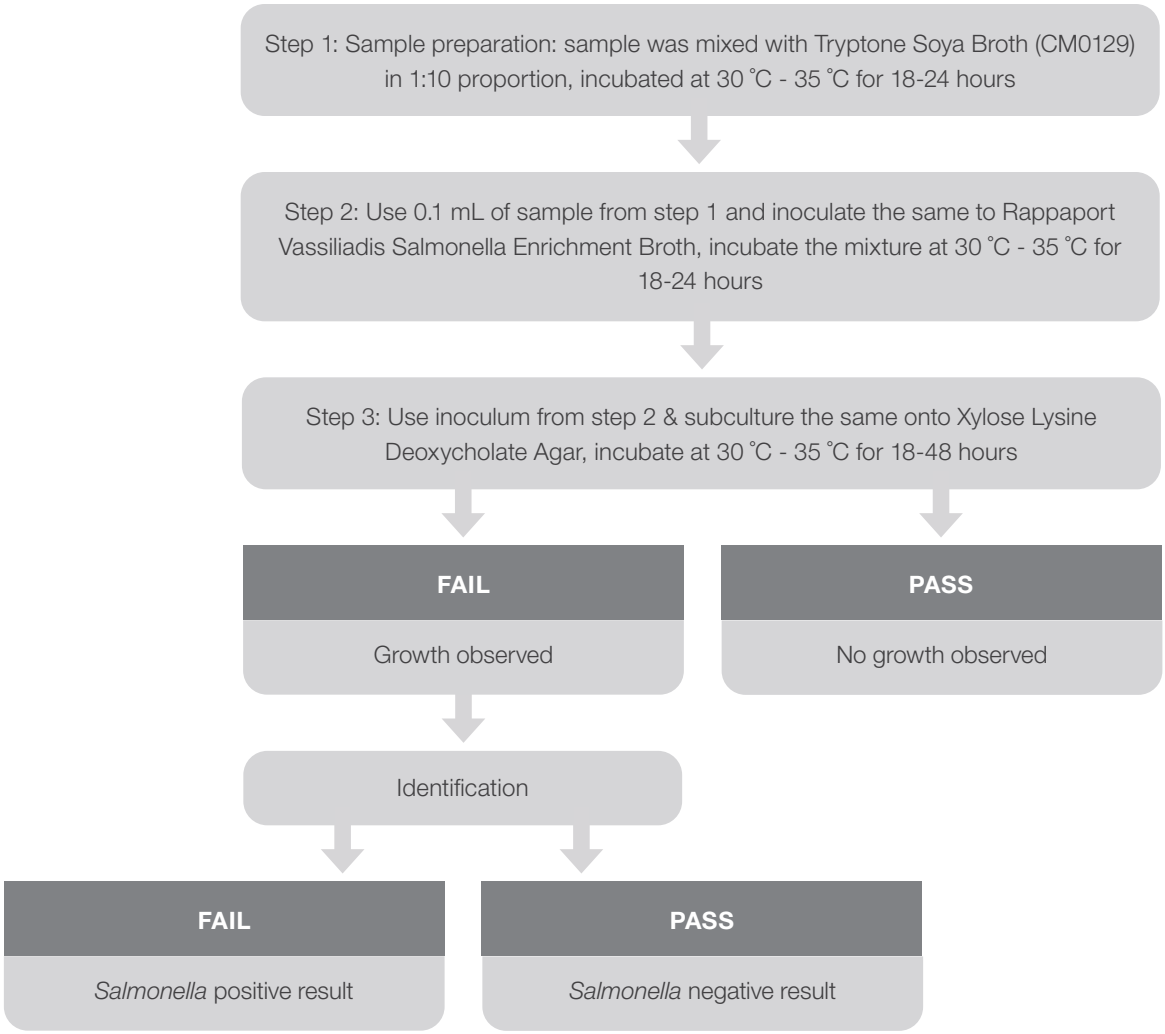
Components	Gram/Litre
Magnesium chloride	13.6
Potassium dihydrogen phosphate	0.6
Sodium chloride	5.0
Dipotassium phosphate	0.40
Soya peptone	4.5
Malachite green	36.0

Mode of action

Most of the enteric Gram-negative bacteria ferment 'xylose' components more rapidly than *Shigella* spp. and produce red colonies. Lysine provides for the differentiation of *Salmonella* spp. from non-pathogenic enteric Gram-negative bacteria. *Salmonella* ferments xylose and produces lysine decarboxylase, resulting in alkaline pH and the formation of red coloured colonies. Sodium thiosulphate and ferric ammonium citrate are added for the detection of enteric Gram-negative bacteria to produce hydrogen sulfide and form black-centered colonies under alkaline conditions. These microbes include *Salmonella* spp., which ferment xylose, lactose or sucrose to provide an acidic pH to the media and produces yellow colonies. These microorganisms are classified as lysine negative. Deoxycholate is a selective agent which inhibits Gram-positive organisms in the media.

Components	Gram/Litre
Lactose	7.5
Yeast extract	3.0
Sucrose	7.5
Sodium deoxycholate	2.50
Sodium thiosulphate	6.8
Ferric ammonium citrate	0.8
L-Lysine	5.0
Phenol red	0.08
Sodium chloride	5.0
Xylose	3.5
Agar	13.5

Test for absence of *Salmonella*



Product	Property	Format	Product code
Absence of *Salmonella Typhimurium*			
Rappaport Vassiliadis Salmonella Enrichment	Broth Nutritive for *S. enterica* spp. Typhimurium	500g	R455432
XLD Agar	Nutritive & indicative for *Salmonella* Typhimurium	Monoplate	R01980
		500g	R459902
Salmonella enterica subsp. *enterica* serovar Typhimurium ATCC® 14028™	Growth promoting of RVS Enrichment Broth Growth promoting & indicative of XLD Agar	Quanti-Cult Plus: 100 tests/kit	R4716000
		Culti-Loops: 5 Loops/pack	R4606000
Salmonella sp. serovar Abony NCTC 6017	Growth promoting of RVS Enrichment Broth Growth promoting & indicative of XLD Agar	Quanti-Cult Plus: 100 tests/kit	R4716007
		Culti-Loops: 5 Loops/pack	R4606007
Staphylococcus aureus ATCC® 6538™	Inhibitory of RVS Enrichment Broth	Quanti-Cult: 10 tests/kit	R4737016
		Quanti-Cult Plus: 100 tests/kit	R4717016
		Culti-Loops: 5 Loops/pack	R4607016

Pseudomonas aeruginosa

As a Gram-negative microorganism, *Pseudomonas aeruginosa* is usually associated with water contamination. It is an opportunistic pathogen and has been linked with severe infections in the eye and wounds caused by burns. It is also very adaptable to its environment and is known to be able to develop resistance to some disinfectants.

Cetrimide Agar

(Pseudomonas Selective Agar, Base)

This medium complies with the recommendations of the harmonized method in the European Pharmacopeia 6.0 and the United States Pharmacopeia 29 (2006).

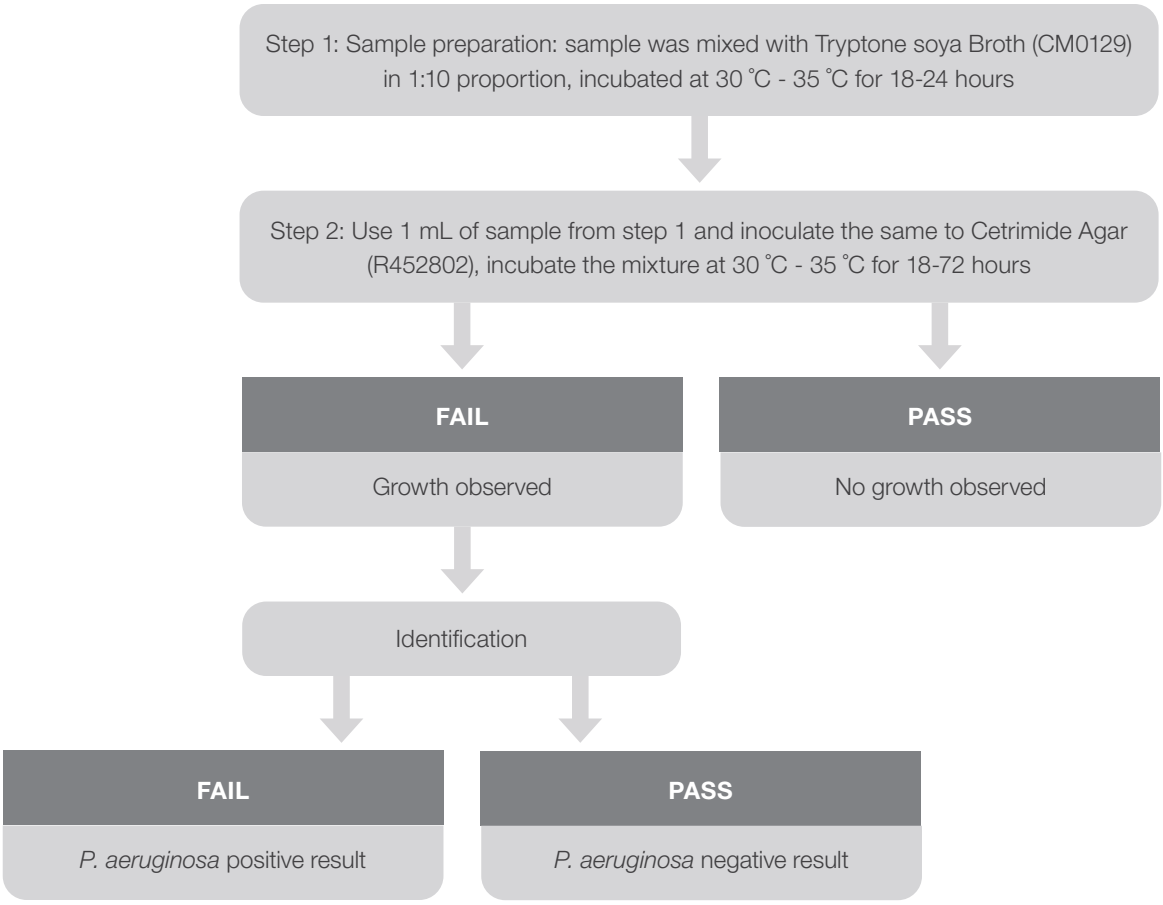
Mode of action

The use of cetrimide (cetyltrimethylammonium bromide) was recommended by Lowbury (1951) and other authors. This compound largely inhibits the growth of the accompanying microbial flora. According to Lowbury and Collins (1955), a concentration of 0.3 g/L inhibits the accompanying organisms satisfactorily and minimizes interference with the growth of *P. aeruginosa*. The pigment production of *P. aeruginosa* is not inhibited when grown on this medium. Goto and Enomoto (1970) recommended the addition of 15 µg/mL nalidixic acid to improve the inhibition of the accompanying microbial flora.

Components	**Gram/Litre**
Peptone from gelatin	20.0
Magnesium chloride	1.4
Potassium sulfate	10.0
N-cetyl-N, N, N-trimethylammoniumbromide (cetrimide)	0.3
Agar-agar	13.6
Malachite green	36.0

Also to be added	**Gram/Litre**
Glycerol	10.0 mL

Test for absence of *Pseudomonas aeruginosa*



Product	Property	Format	Product code
Test for *Pseudomonas aeruginosa*			
Cetrimide Agar	Nutritive for *P. aeruginosa* & selective for *E. coli*	Monoplate	R01292
		500g	R452802
Pseudomonas aeruginosa ATCC® 9027™	Growth promoting of Cetrimide Agar	Quanti-Cult: 10 tests/kit	R4735210
		Quanti-Cult Plus: 100 tests/kit	R4715210
		Culti-Loops: 5 Loops/pack	R4605210
Escherichia coli ATCC® 8739™	Inhibitory of Cetrimide Agar	Quanti-Cult: 10 tests/kit	R4737085
		Quanti-Cult Plus: 100 tests/kit	R4717085
		Culti-Loops: 5 Loops/pack	R4607085

Staphylococcus aureus

Staphylococcus aureus is a human skin commensal and, if present, is highly likely to be associated with operator-associated contamination. It is undesirable as at high levels (105 CFU/g), it is capable of producing an endotoxin. The toxin is heat stable and can cause severe effects, such as stomach cramps and severe vomiting. Dehydration may also be a problem. *Staphylococcus aureus* is an opportunistic pathogen and can cause severe systemic infections, such as meningitis. It can also be the infective agent for skin lesions and can cause spots and boils. Although not as severe as *Salmonella* spp., the effects are undesirable consequences from the ingestion of a medicinal product.

Mannitol Salt Phenol-red Agar

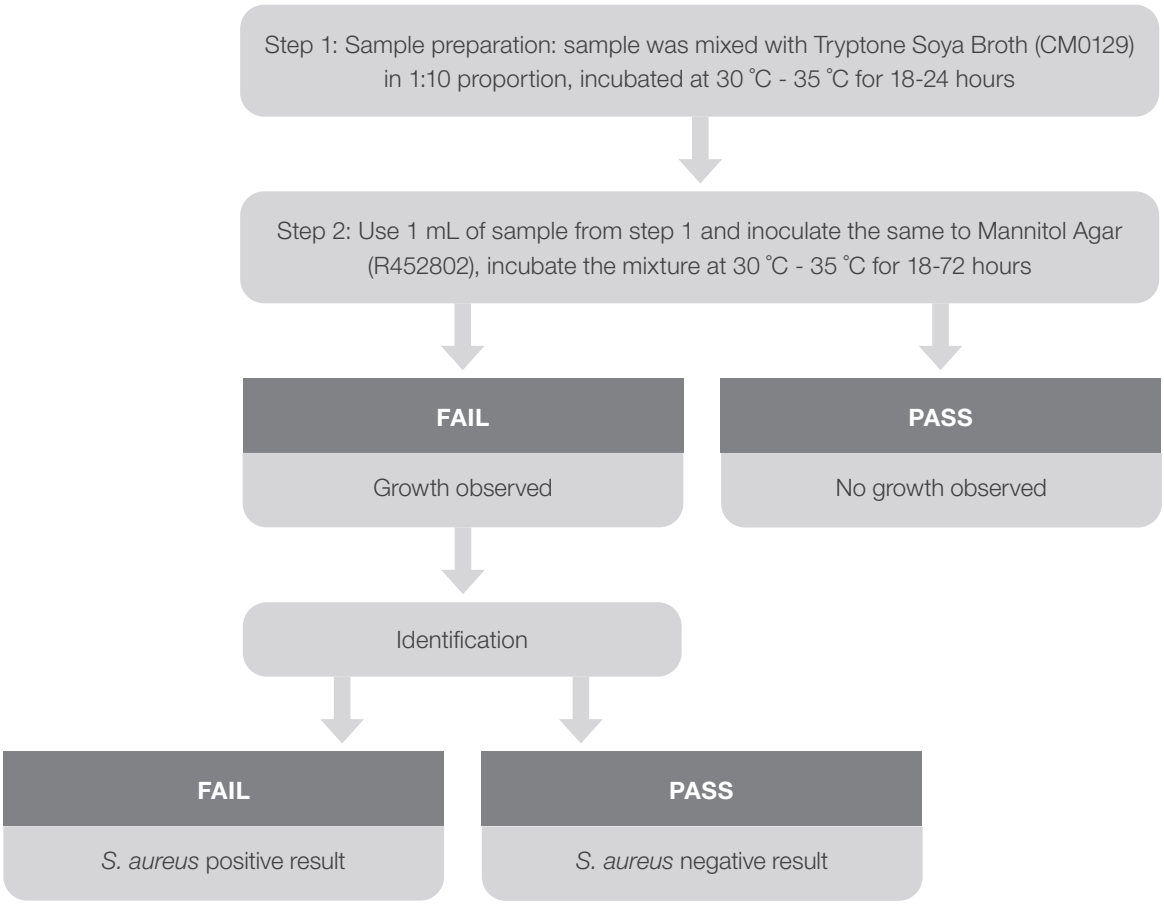
In 1942, Koch reported the use of 7.5% sodium chloride as a selective agent for the isolation of staphylococci. Chapman confirmed the results of Koch and suggested the addition of 7.5% sodium chloride to phenol-red mannitol agar. Most strains of coagulase-positive staphylococci grow on Mannitol Salt Agar, producing yellow zones as a result of mannitol fermentation. Coagulase-negative strains of staphylococci produce small colonies with red-colored zones in the surrounding medium.

Components	**Gram/Litre**
Sodium chloride	75.0
Beef extract	1.0
D-mannitol	10.0
Phenol red	25.0
Casein peptone	5.0
Agar	15.0
Meat peptone	5.0
Demineralized water	1000.0

Mode of action

Casein and meat peptones supply nitrogen, amino acids, and peptides necessary for bacterial growth. Sodium chloride in a concentration of 7.5% is a selective agent which inhibits many bacteria other than staphylococci. Phenol red is a pH indicator which causes a color change in the medium from red-orange to yellow when acid is produced. Staphylococci colonies that ferment mannitol will be surrounded by a yellow zone, while those that do not ferment mannitol will have a red zone.

Test for absence of *Staphylococcus aureus*



Product	Property	Format	Product code
Test for *Staphylococcus aureus*			
Mannitol Salt Agar	Nutritive for *S. aureus* & selective for *E. coli*	Monoplate	R01580
		500g	R453902
Staphylococcus aureus ATCC® 6538™	Growth promoting & indicative of Mannitol Salt Agar	Quanti-Cult: 10 tests/kit	R4737016
		Quanti-Cult Plus: 100 tests/kit	R4717016
		Culti-Loops: 5 Loops/pack	R4607016
Escherichia coli ATCC® 8739™	Inhibitory of Mannitol Salt Agar	Quanti-Cult: 10 tests/kit	R4737085
		Quanti-Cult Plus: 100 tests/kit	R4717085
		Culti-Loops: 5 Loops/pack	R4607085

Clostridia

Reinforced Clostridial Medium (RCM)

Hirsch and Grinstead developed Reinforced Clostridial Medium (RCM) for the cultivation and enumeration of clostridia. Reinforced Clostridial Medium MLT is a nonselective enrichment medium that supports the growth of various anaerobic and facultative bacteria when incubated anaerobically. It is formulated in conformance with harmonized United States Pharmacopeia (USP)/European Pharmacopeia (EP) guidelines for use in testing for the presence of *Clostridium* spp.

Mode of Action

Peptone and beef extract are sources of carbon, nitrogen, vitamins, and minerals essential for bacterial growth. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Dextrose is an energy source. Sodium chloride maintains osmotic equilibrium. Sodium acetate is a buffering agent. Starch acts as a protective colloid against toxic materials present in the medium. Cysteine hydrochloride is a reducing agent. A small amount of agar is added to impede the diffusion of oxygen.

Components	Gram/Litre
Beef extract	10.0
Yeast extract	3.0
Peptone	10.0
Soluble starch	1.0
Dextrose	5.0
Cysteine hydrochloride	0.5
Sodium chloride	5.0
Agar	0.5
Sodium acetate	3.0
Demineralized water	1000.0

Columbia Agar (Base)

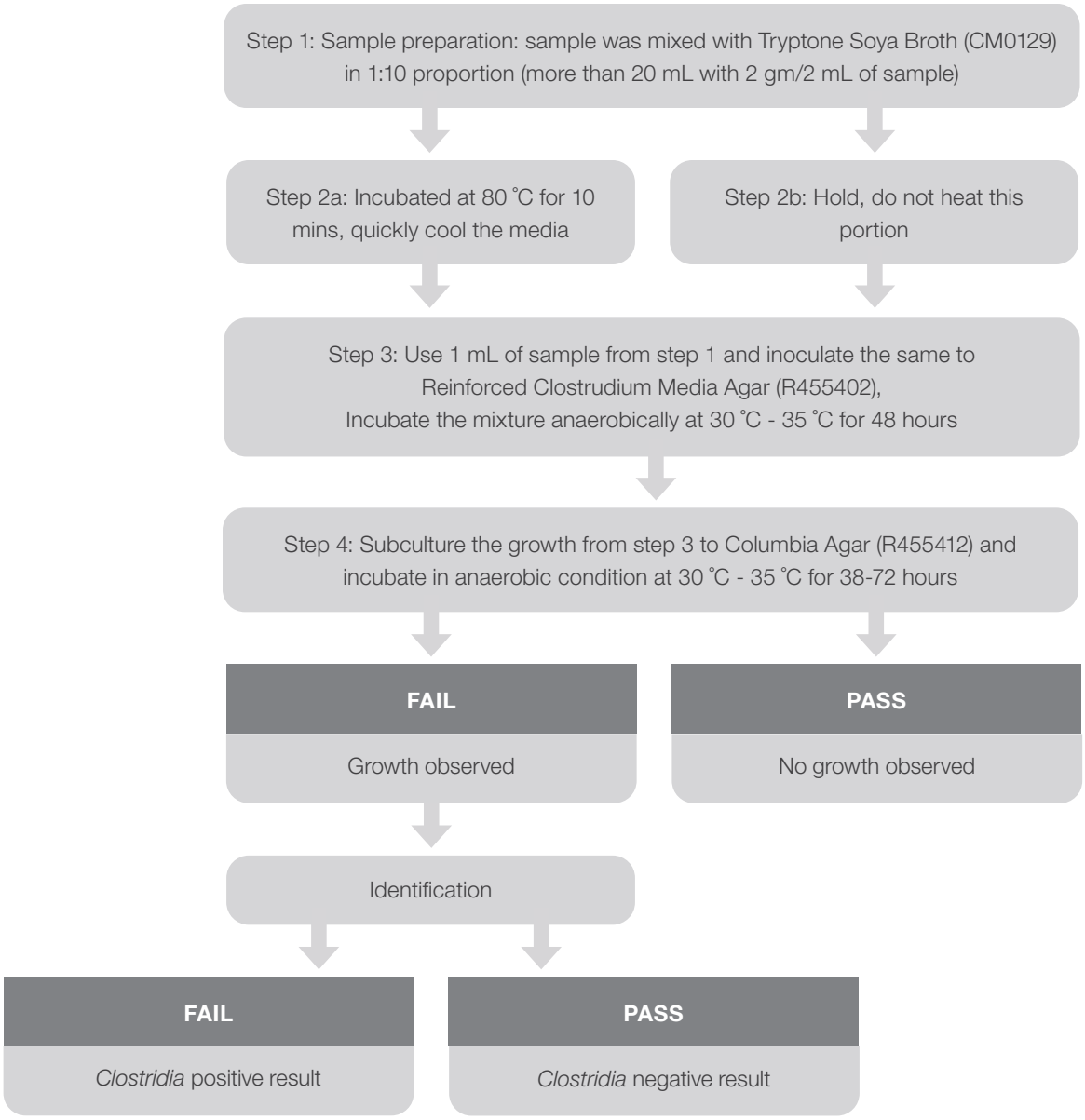
Columbia Agar was developed by Ellner et al. at Columbia University. Prior to that time, traditional bases were made from either casein hydrolysate or meat infusion media. Ellner combined peptones from both animal and vegetable proteins, resulting in a base that supports the growth of both fastidious and nonfastidious organisms. Columbia Agar MLT is formulated in conformance with harmonized United States Pharmacopeia (USP)/ European Pharmacopeia (EP) guidelines for use in testing for the presence of *Clostridium* spp.

Mode of Action

Peptones supply growth factors such as nitrogen, carbon, vitamins, and trace elements essential for bacterial growth. Corn starch serves as an energy source and yeast extract supplies B-complex vitamins.

Components	Gram/Litre
Pancreatic digest of casein	10.0
Heart pancreatic digest	3.0
Meat peptic digest	5.0
Corn starch	1.0
Sodium chloride	5.0
Agar	12.5
Yeast extract	5.0
Demineralized water	1000.0

Test for absence of *Clostridia*



Product	Property	Format	Product code
Test for Clostridia			
Reinforced Clostridial Medium	Non-selective enrichment	500 g	R455402
		100 mL	R112548
Clostridium sporogenes ATCC® 11437™	Growth promoting of RCM and Columbia Agar	Quanti-Cult: 10 tests/kit	R4731703
		Quanti-Cult Plus: 100 tests/kit	R4711703
		Culti-Loops: 5 Loops/pack	R4601703
Clostridium sporogenes ATCC® 19404™	Growth promoting of RCM and Columbia Agar	Quanti-Cult Plus: 100 tests/kit	R4711700
		Culti-Loops: 5 Loops/pack	R4601700

Candida albicans

Sabouraud - 2% Dextrose Broth

Sabouraud Dextrose Broth was described by Sabouraud in 1892. Emmons modified Sabouraud's formulation by reducing the dextrose from 40 g/L to 20 g/L. Sabouraud Dextrose Broth (2%) is formulated in conformance with harmonized United States Pharmacopeia (USP)/European Pharmacopeia (EP) guidelines.

Mode of Action

Casein and meat peptones supply nitrogenous compounds and amino acids necessary for the growth of yeasts and fungi. Dextrose is a ready source of energy. The low pH of the medium is favorable to the growth of fungi, especially dermatophytes, while also inhibiting bacteria.

Components	Gram/Litre
Dextrose	20.0 g
Lactose	5.0 g
Meat peptone	5.0 g
Demineralized water	1000.0 mL

Sabouraud Dextrose Agar pH 5.6 w/ and w/o Chloramphenicol

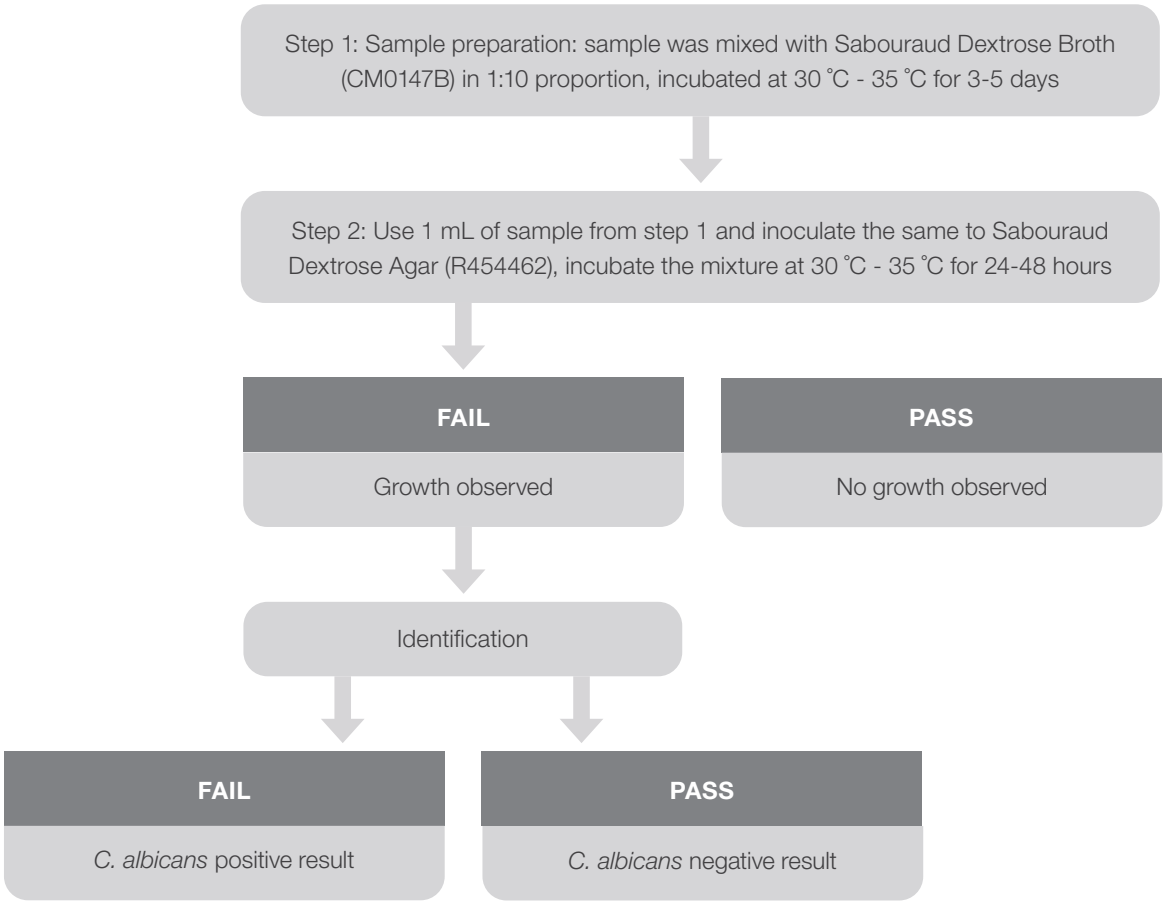
Sabouraud Dextrose Agar was developed by Sabouraud in 1892 for cultivation of dermatophytes. The low pH of 5.6 enhances the growth of fungi, especially dermatophytes, and is slightly inhibitory to bacteria in clinical specimens. This medium is recommended by the U.S. Pharmacopeia for mold and yeast counts. It is also recommended by the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). The addition of chloramphenicol to the base agar makes the medium more selective.

Mode of Action

Casein and meat peptones provide nitrogen, amino acids, and peptides necessary for the growth of fungi. Dextrose is an energy source. Chloramphenicol is a selective agent which is inhibitory to most bacteria.

Components	Gram/Litre
Casein peptone	5.0 g
Dextrose	40.0 g
Meat peptone	5.0 g
Agar	15.0 g
Demineralized water	1000.0 mL

Test for absence of *Candida albicans*



Product	Property	Format	Product code
Test for *Candida albicans*			
Sabouraud Dextrose Broth	Nutritive for *C. albicans*	15 x 103 mm tube, 5mL	R064410
		100 mL screw cap bottle	R112653
		100 mL screw cap bottle, double bagged	R112553
Sabouraud Dextrose Agar 5.6	Nutritive & indicative for *C. albicans*	500 mL	R112551
		100 mL agar	BO1155M
		250 mL agar	BO1155T
		Agar plate	R112550
		500 g	R454462
Candida albicans ATCC® 10231™	Growth promoting of Sabouraud Dextrose Broth Growth promoting & indicative of Sabouraud Dextrose Agar	Quanti-Cult: 10 tests/kit	R4731503
		Quanti-Cult Plus: 100 tests/kit	R4711503
		Culti-Loops: 5 Loops/pack	R4601503

Burkholderia cepacia

This is a Gram-negative aerobic bacterium that is widespread and commonly found in soil and water. It is capable of surviving for extended periods of time in hostile environments due to its innate resistance to many antiseptics and antibiotics.

BCSA

Initially a newly developed *B. cepacia* selective agar (BCSA), which was more selective against other organisms than currently available selective agars, was developed. The addition of vancomycin to this new medium made it similar to oxidation-fermentation polymyxin-bacitracin-lactose (OFPBL) medium. The vancomycin reduced the number of false positives and increased selectivity of the medium. This new selective media was for patients with Cystic Fibrosis. Another outbreak of *B. cepacia* due to use of OTCs and other aqueous solutions occurred and another BCSA was needed. The BCSA for CF patients was adjusted to test finished goods and not patient samples. USP 60 provided a new regulation for non-sterile products and a minor pH adjustment with the existing formula satisfied regulations.

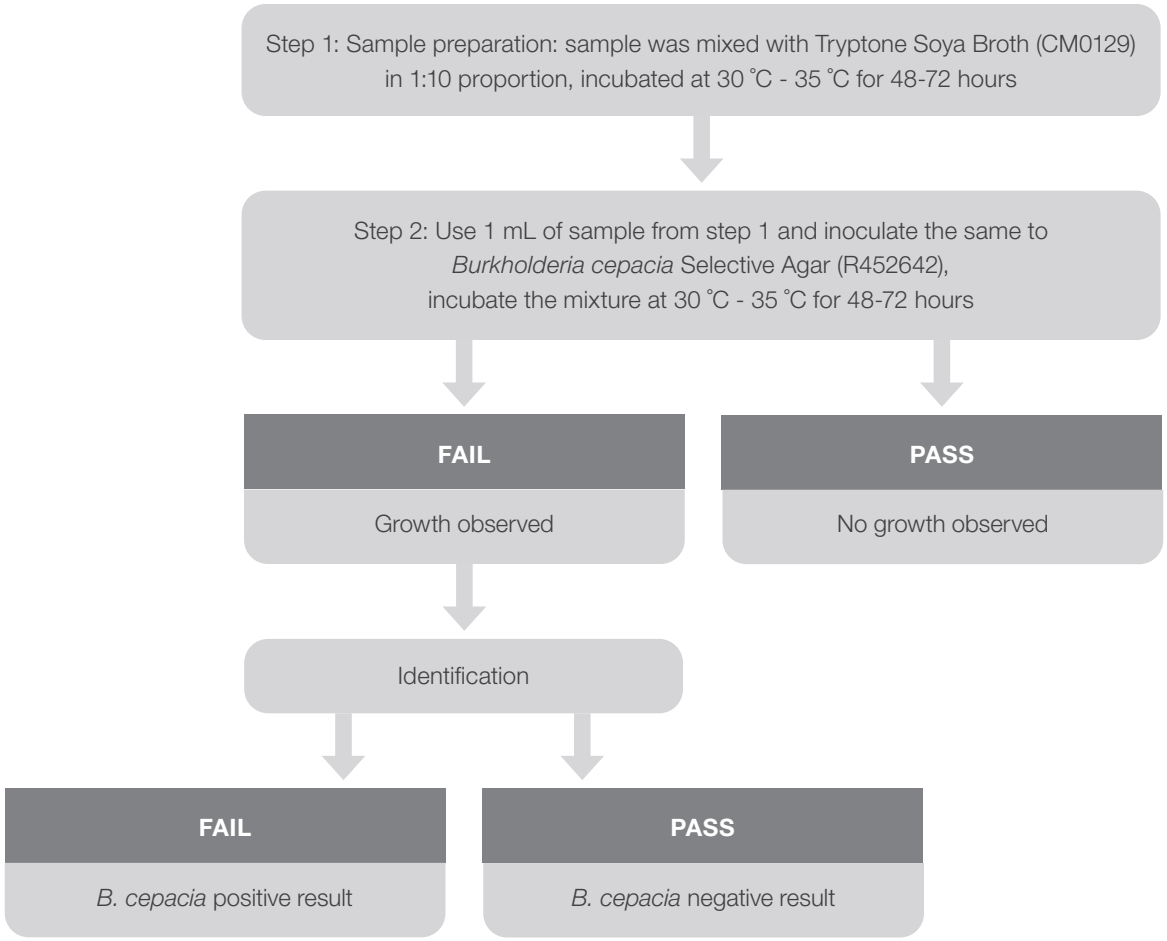
Components	**Gram/Litre**
Casein peptone	10.0 g
Lactose	10.0 g
Sucrose	10.0 g
Sodium chloride	5.0 g
Yeast extract	1.5 g
Phenol red	0.08 g
Gentamicin	10.0 mg
Vancomycin	2.5 g
Crystal violet	2.0 mg
Polymyxin B	600.000 U
Agar	14.0 g
Demineralized water	1000.0 mL

Mode of Action

The plate oxidizes and ferments sugars and/or oxidation of *lysine decarboxylase*; (ii) weakly positive oxidase reaction, defined as a faint purple to pink color.

Note: Antibiotics are added separately to manually prepared medium. Calculations are required.
Supplements: Thermo Scientific™ SR0247E, SR0186E Oxoid™ Vancomycin Supplement Thermo Scientific™
SR0099E Oxoid™ Bacillus Cereus Selective Supplement (Polymyxin B)
Thermo Scientific™SR0185E Oxoid™ Gentamicin Selective Supplement

Test for absence of *Burkholderia cepacia*



Product	Property	Format	Product code
Test for *Burkholderia cepacia*			
BCSA agar	Selective and differential media for *B. cepacia*	10/pk	R110245 R110244 R452642
Burkholderia cepacia ATCC® 25416™	Growth promoting & indicative of BCSA Agar	Quanti-Cult Plus: 100 tests/kit	R4715220
		Culti-Loops: 5 Loops/pack	R4605220
Burkholderia cenocepacia ATCC® BAA-245™	Growth promoting & indicative of BCSA Agar	Quanti-Cult Plus: 100 tests/kit	R4715221
Burkholderia multivorans ATCC® BAA-247™	Growth promoting & indicative of BCSA Agar	Quanti-Cult Plus: 100 tests/kit	R4715222
Pseudomonas aeruginosa ATCC® 9027™	Inhibitory of BCSA	Quanti-Cult: 10 tests/kit	R4735210
		Quanti-Cult Plus: 100 tests/kit	R4715210
		Culti-Loops: 5 Loops/pack	R4605210
Staphylococcus aureus ATCC® 6538™	Inhibitory of BCSA	Quanti-Cult: 10 tests/kit	R4737016
		Quanti-Cult Plus: 100 tests/kit	R4717016
		Culti-Loops: 5 Loops/pack	R4607016

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