thermo scientific

Pharmaceutical microbiology

Globally harmonized EP/USP/JP

ThermoFisher SCIENTIFIC

Contents

This guide contains consolidated algorithms provided by Dr. Scott Sutton, as an outline for detection of specific pathogens isolated in the pharmaceutical microbiology laboratory.

For more detailed information, please consult the appropriate U.S. Pharmacopeia methods.

Introduction

Whether performing testing for microbial limits, sterility, environmental monitoring or *Mycoplasma*: companies need a partner that can deliver science, service, and confidence to get the job done.

Utilizing the latest technology in our expanded, state-of-the-art manufacturing facility, and backed by a team of experts dedicated to microbiology, we deliver the high-performance pharmaceutical microbiology products your laboratory can depend on, for reliable results you can trust.

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Manufactured in our FDA and ISO-certified facilities, Thermo Scientific™ Remel™ and Oxoid™ media products are specially formulated to meet or exceed USP specifications.

- Each lot number tested for uniformity and consistency, for ultimate performance
- Manufactured in controlled environmental conditions, to ensure product stability
- Flexible standard sizes, including 500 g, 2.5 kg, 5 kg, and 10 kg
- Custom formulations available upon request, to meet your laboratory's unique needs

Environmental testing

Our extensive line of Thermo Scientific™ Isolator Wrap™ sterile contact plates and sterile settling plates are manufactured in a Class 10,000 (ISO Class 7) clean room with Class 100 (ISO Class 5) work zones, and gamma-irradiated to a sterility assurance level (SAL) of >10-6, for reliable, consistent environmental testing.

- Sterile contact and settling plates are double-bagged, to preserve specimen quality, with a third bag for specimen transport
- Isolator Wrap sterile contact plates have a six-month shelf-life, and are packaged in a protective outer barrier, impermeable to VHP, IPA or bleach. Includes extra bag for specimen transport.
- Thermo Scientific™ GripFit™ plates ensure the lid remains on the plate, for enhanced safety, and are easy to remove
- Thermo Scientific™ Triple Wrap sterile contact and settling plates have a nine-month shelf-life, room temperature storage, clear packaging with unique VHP indicator and bi-plex polypropylene shipping container. No VHP breakthrough or cardboard contaminants coming in contact with delicate media for safe, sterile, secure confidence.

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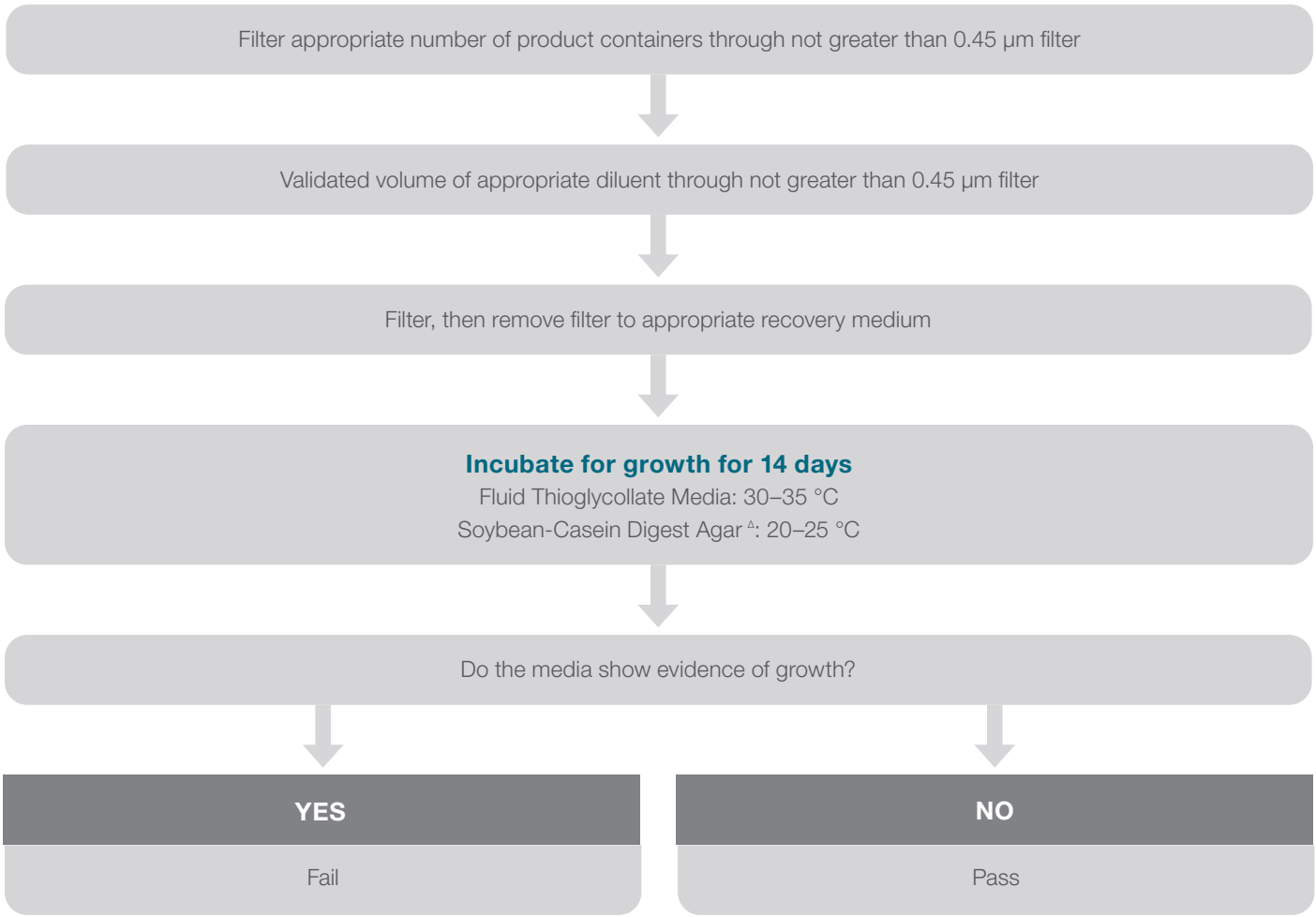
We offer one of the most extensive quality control product lines available, including a wide range of ATCC® organisms in easy-to-use formats, for consistent, accurate pharmaceutical testing.

- Thermo Scientific™ Culti-Loops® are ready-to-use, disposable inoculation loops containing stabilized, preserved, viable microorganisms, for simple and convenient performance testing
- Thermo Scientific™ Quanti-Cult® products are pre-quantitated to deliver <100 CFUs, saving you time, money and reducing procedure time from 2-3 days to 15 minutes
- Thermo Scientific™ WaterBugs™ and WKITS™ are environmental QC microorganisms that simulate actual water/waste-water samples. This Quanti-Cult format (<50 CFUs) saves you time, money, and reduces procedure time from 2-3 days to 15 minutes.

Renowned for quality, accuracy, reliability and innovation, we bring you full access to superior products and service that can only come from being part of Thermo Fisher Scientific. Trust Thermo Scientific Pharmaceutical Microbiology Solutions to deliver the science, service, and confidence you need to get your job done.

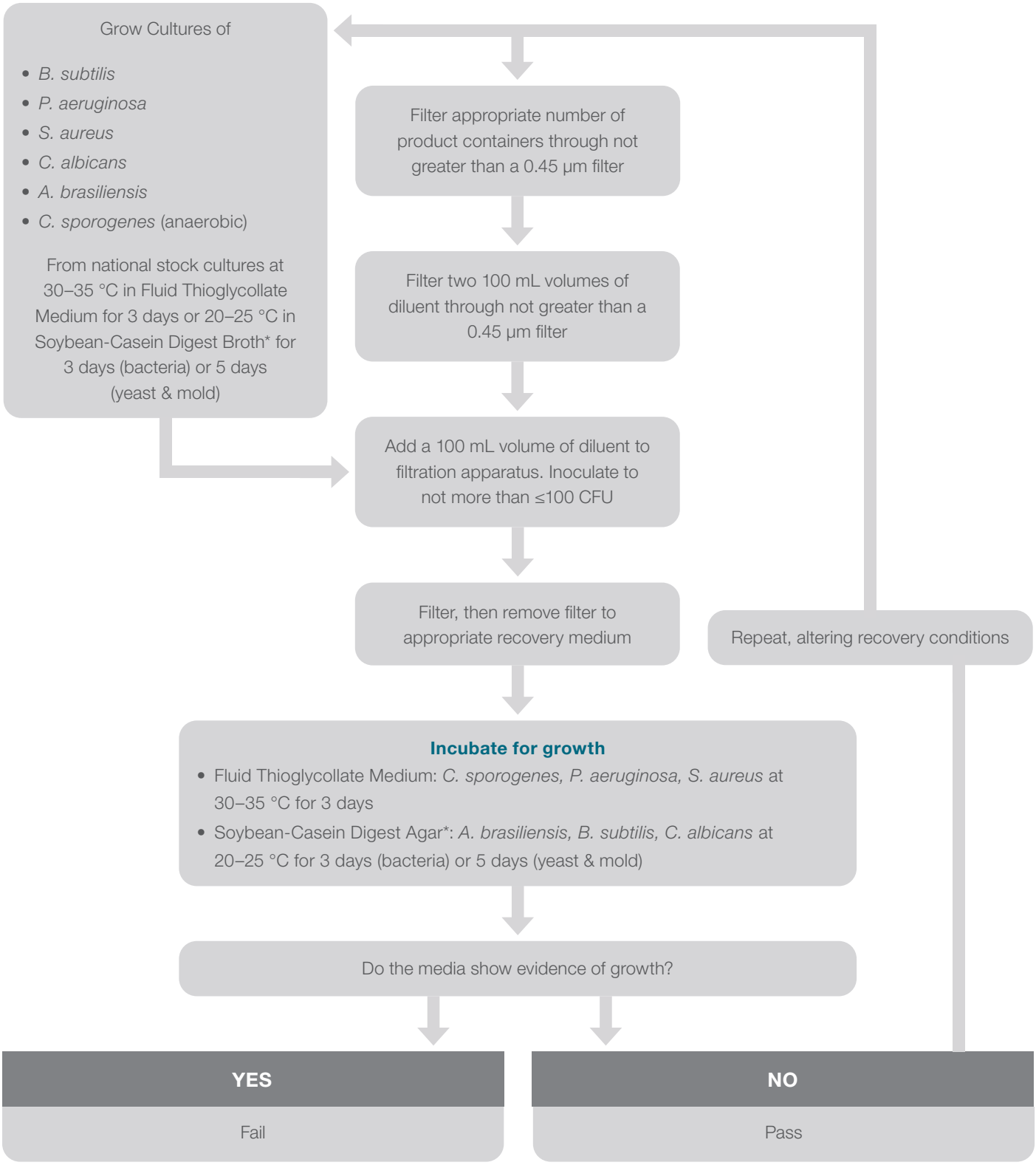
Sterility Test: Membrane Filtration



[A] Soybean-Casein Digest Broth = Tryptic Soy Broth (TSB)

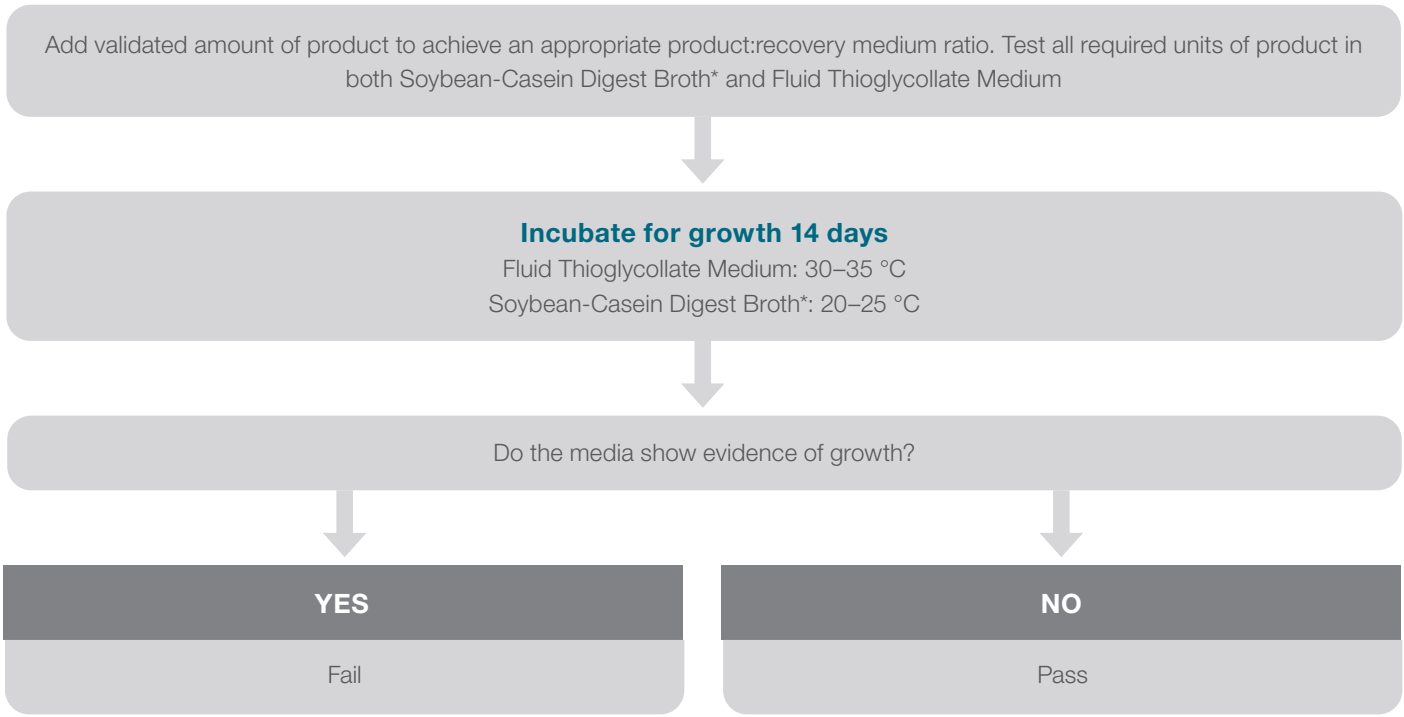
Sterility Test: Method Suitability Study Design

Membrane Filtration



[A] Soybean-Casein Digest Broth = Tryptic Soy Broth (TSB)

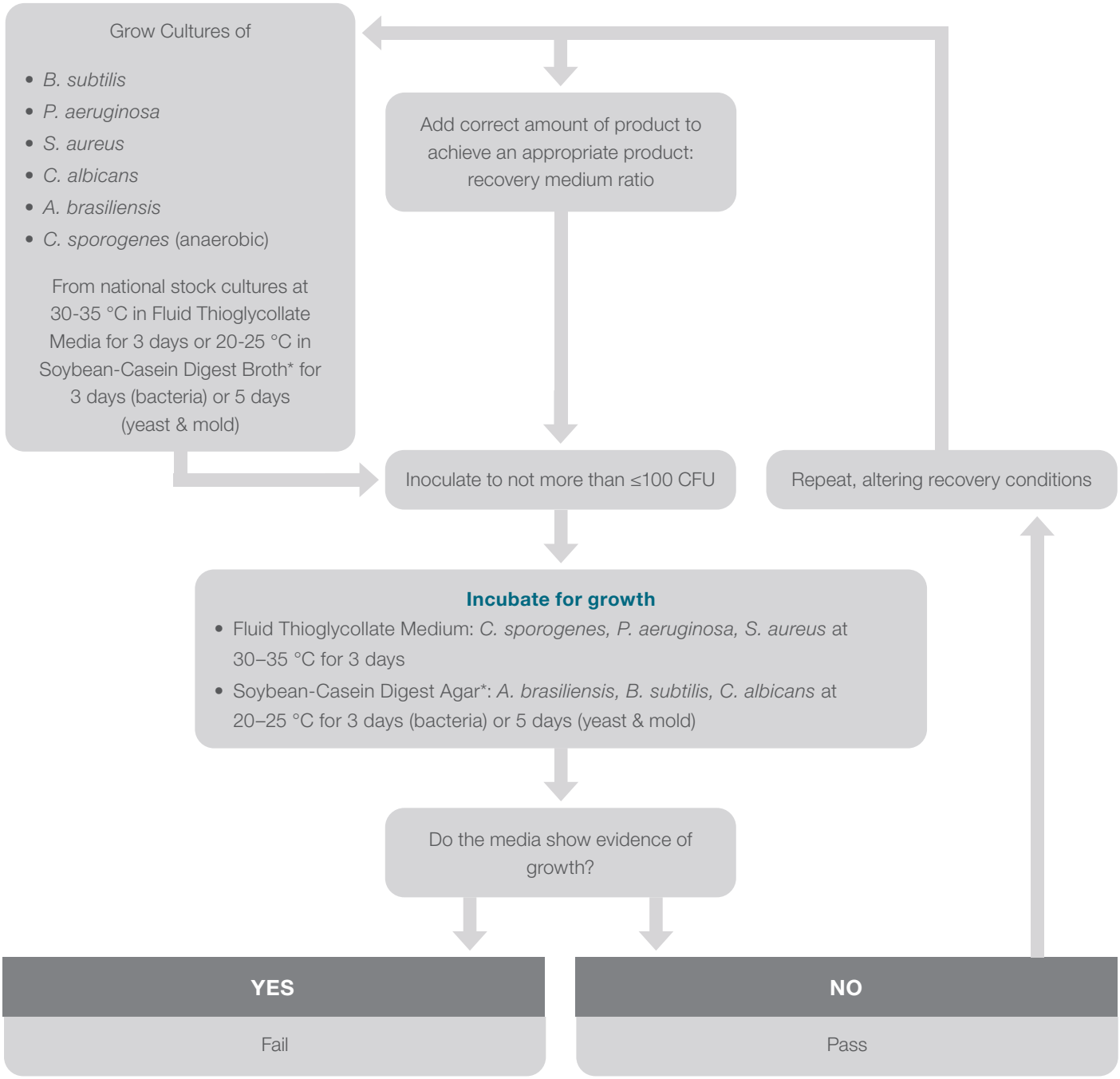
Sterility Test: Direct Transfer



[a] Soybean-Casein Digest Broth = Tryptic Soy Broth (TSB)

Sterility Test: Method Suitability Study Design

Direct Transfer



[A] Soybean-Casein Digest Broth = Tryptic Soy Broth (TSB)

Sterility Testing: Diluting and rinsing fluids

Sampling and Growth

Product	Format	Product Code
Fluid Thioglycollate Medium	10 mL tube, 20 /pk	R07174
	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
	9 mL tube	TV5001D
Fluid Thioglycollate Medium, Dehydrated	500 g	R453452
	2.5 kg	R453454
Tryptic Soy Broth (TSB)*	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	BO351U
	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

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
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

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	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
Storage bag for triple wrap settling plate	Cellophane Bags	10 p/k	R111551
Swabs	Sani-Cult™	5 mL, 100 /pk	R723141

† Not intended for IVD use

Mycoplasma Transport and Isolation

Product	Format	Product code
MicroTest™ M4®	3 mL tube, 12 /pk	R12502
	3 mL tube, 72 /pk	R12500
MicroTest™ M5®	3 mL tube, 12 /pk	R12516
	3 mL tube, 72 /pk	R12515
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
PPLO Agar/Broth	5 mL tube	R20360
Mycoplasma Broth Base	500 g	R454172
Mycoplasma Broth, Frey	500 g	R454162

Quality Control Organisms

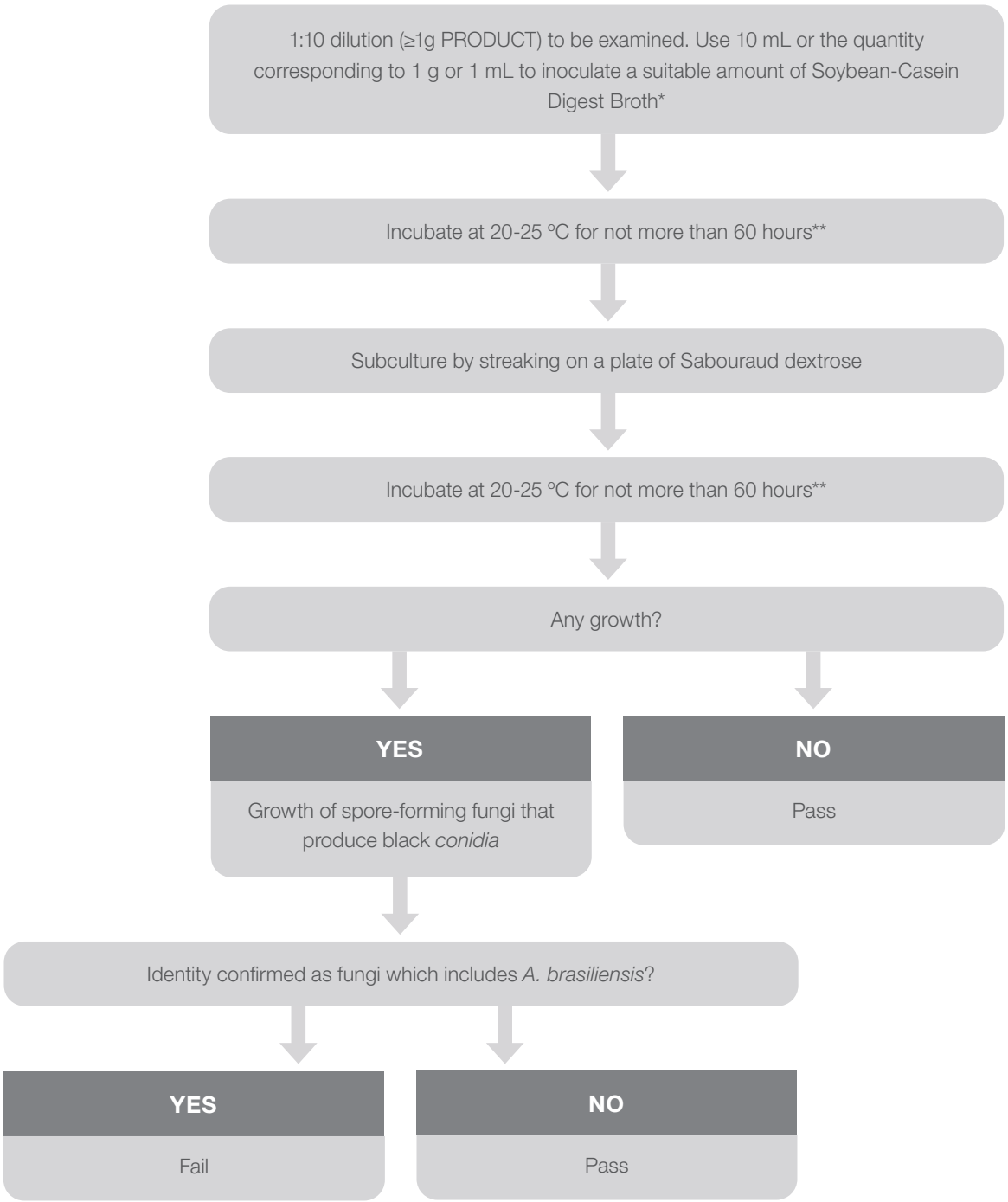
Product	Format	Product code
Aspergillus brasiliensis ATCC® 16404™	Quanti-Cult: 10 tests/kit	R4731100
	Quanti-Cult Plus: 100 tests/kit	R4711100
	Culti-Loops: 5 Loops/pack	R4601100
Bacillus subtilis ATCC® 6633™	Quanti-Cult: 10 tests/kit	R4731221
	Quanti-Cult Plus: 100 tests/kit	R4711221
	Culti-Loops: 5 Loops/pack	R4601221
Burkholderia cenocepacia ATCC® BAA-245™	Quanti-Cult Plus: 100 tests/kit	R4715221
Burkholderia cepacia ATCC® 25416™	Quanti-Cult Plus: 100 tests/kit	R4715220
	Culti-Loops: 5 Loops/pack	R4605220
Burkholderia multivorans ATCC® BAA-247™	Quanti-Cult Plus: 100 tests/kit	R4715222
Candida albicans ATCC® 10231™	Quanti-Cult: 10 tests/kit	R4731503
	Quanti-Cult Plus: 100 tests/kit	R4711503
	Culti-Loops: 5 Loops/pack	R4601503
Clostridium sporogenes ATCC® 11437™	Quanti-Cult: 10 tests/kit	R4731703
	Quanti-Cult Plus: 100 tests/kit	R4711703
	Culti-Loops: 5 Loops/pack	R4601703
Clostridium sporogenes ATCC® 19404™	Quanti-Cult Plus: 100 tests/kit	R4711700
	Culti-Loops: 5 Loops/pack	R4601700
Escherichia coli ATCC® 8739™	Quanti-Cult: 10 tests/kit	R4737085
	Quanti-Cult Plus: 100 tests/kit	R4717085
	Culti-Loops: 5 Loops/pack	R4607085
Kocuria rhizophila ATCC® 9341™	Quanti-Cult Plus: 100 tests/kit	R4714075
	Culti-Loops: 5 Loops/pack	R4604075
Pseudomonas aeruginosa ATCC® 9027™	Quanti-Cult: 10 tests/kit	R4735210
	Quanti-Cult Plus: 100 tests/kit	R4715210
	Culti-Loops: 5 Loops/pack	R4605210
Salmonella enterica subsp. *enterica* serovar *Typhimurium* ATCC® 14028™	Quanti-Cult Plus: 100 tests/kit	R4716000
	Culti-Loops: 5 Loops/pack	R4606000
Salmonella sp. serovar Abony NCTC 6017	Quanti-Cult Plus: 100 tests/kit	R4716007
	Culti-Loops: 5 Loops/pack	R4606007
Staphylococcus aureus ATCC® 6538™	Quanti-Cult: 10 tests/kit	R4737016
	Quanti-Cult Plus: 100 tests/kit	R4717016
	Culti-Loops: 5 Loops/pack	R4607016

Environmental Monitoring

Category	Product	Format	Product code
Isolator Wrap™ Sterile Contac 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

Absence of *Aspergillus brasiliensis*

Aspergillus brasiliensis is a spore producing fungi that produces black conidia (spores) that are readily dispersed in the environment.



* Soybean-Casein Digest Broth = Tryptic Soy Broth (TSB)

**Verify through validated methods the proper incubation timeframe that prevents too numerous to count.

Absence of *Aspergillus brasiliensis*

Test	Product	Property	Format	Product code
Test for A. brasiliensis	Sabouraud Dextrose Broth	Nutritive for A. brasiliensis	15x103mm 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 A. brasiliensis	500mL	R112551
			Agar plate	R112550
			500g	R454462
	Aspergillus brasiliensis ATCC® 16404™	Quantitative QC	Quanti-Cult: 10 tests/kit	R4731100
			Quanti-Cult Plus: 100 tests/kit	R4711100
		Qualitative QC	Culti-Loops: 5 Loops/pack	R4601100

More products available globally-see website or local representative

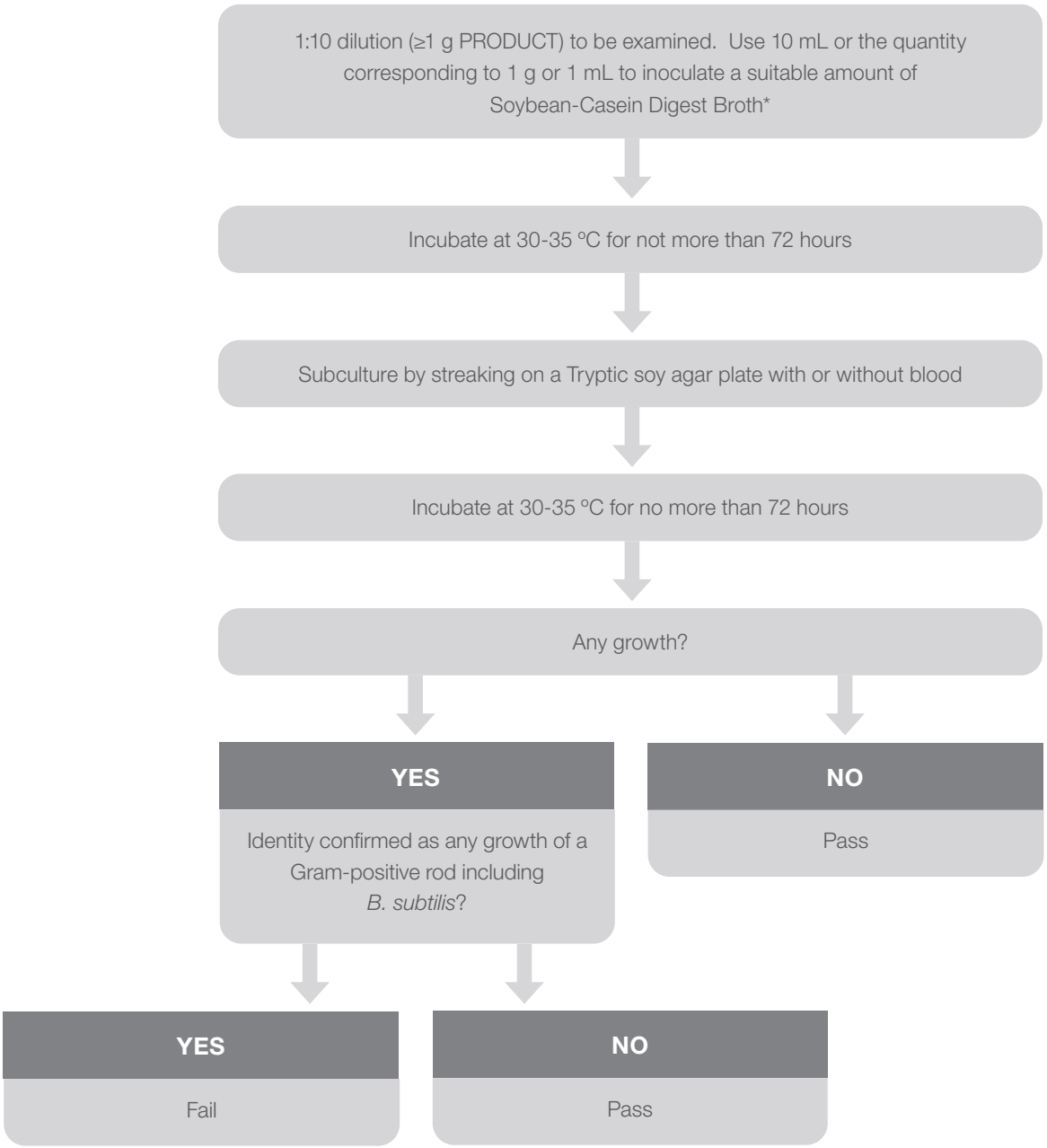
Sabouraud Dextrose Agar pH 5.6 w/ and w/o Chloramphenicol

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.

Classical Formula

Casein peptone	5.0 g
Dextrose	40.0 g
Meat peptone	5.0 g
Agar	15.0 g
Demineralized water	1000.0 mL

Absence of *Bacillus subtilis*



* Soybean-Casein Digest Broth = Tryptic Soy Broth (TSB)

Absence of *Bacillus subtilis*

Bacillus subtilis is a spore forming, motile, rod-shaped, Gram-positive, facultative aerobe. It is mostly found in soil and vegetation with an optimal growth temperature from 25-35 ºC. *B. subtilis* has the ability to produce and secrete antibiotics.

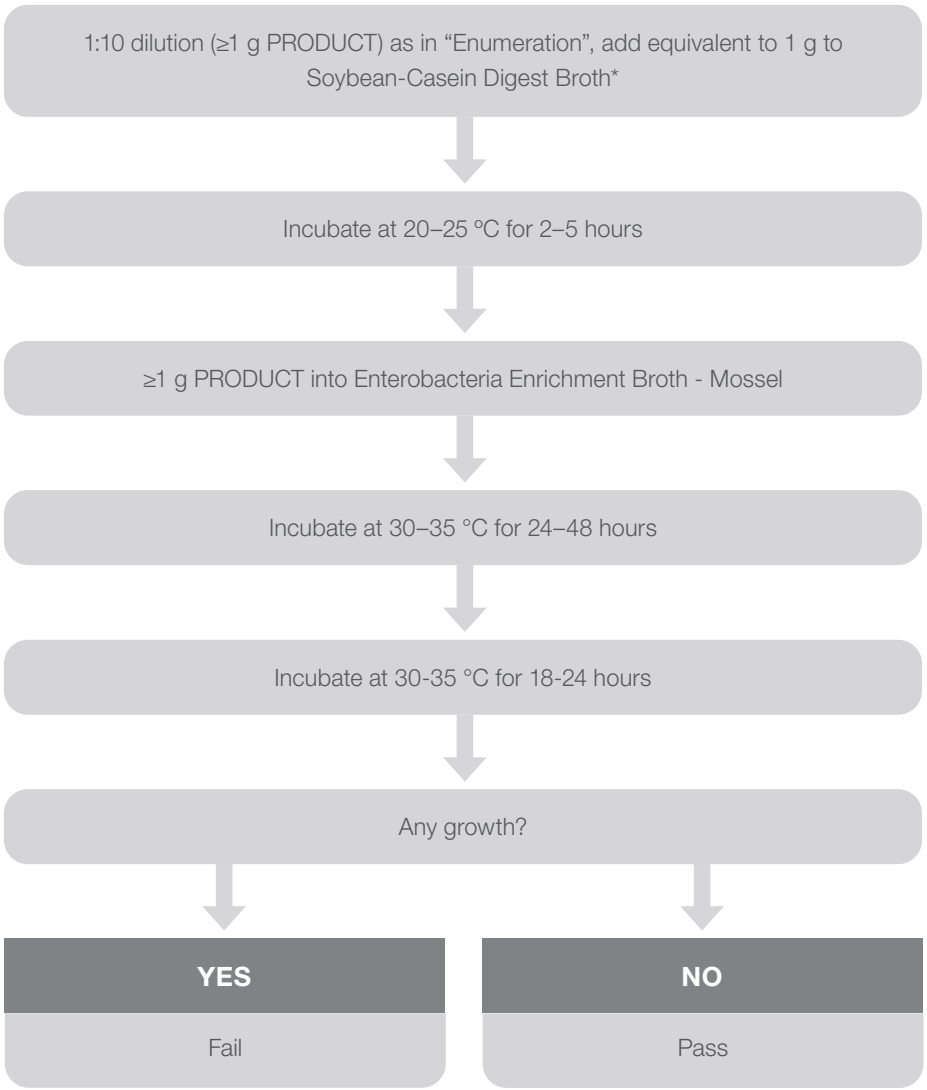
Test	Product	Property	Format	Product code
Test for *B. subtilis*	Tryptic soy broth	Nutritive media	Tryptone Soya Broth (acc. to EP/ USP)	BO0351
	Tryptic soy agar	Nutritive media	Tryptone Soya Agar 5 kg	CM0131T
			TSA (40 mL) 100 /pk	R110092
			TSA (USP) 10 /pk	R110093
			TSA (USP) 100/pk	R110094
			TSA 10 kg each	R455006
			TSA 2.5 kg each	R455004
			TSA 500 g each	R455002
			TSA (500 mL) 10 /pk	R112700
	Bacillus subtilis ATCC® 6633™	Quantitative QC	Quanti-Cult: 10 tests/kit	R4731221
			Quanti-Cult Plus: 100 tests/kit	R4711221
		Qualitative QC	Culti-Loops: 5 Loops/pack	R4601221

More products available globally-see website or local representative

Absence of *B. subtilis*

This organism does not have a selective characteristic and is not required for identification. It is ruled out with the general absence of growth.

Absence of Bile-tolerant Gram-negative Bacteria



* Soybean-Casein Digest Broth = Tryptic Soy Broth (TSB)

Absence of Bile-tolerant Gram-negative Bacteria

These microorganisms are usually associated with aqueous environments and are indicators 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.

Test	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 EE Mossel 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 EE Mossel 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 EE Mossel broth*	Quanti-Cult: 10 tests/kit	R4737016
			Quanti-Cult Plus: 100 tests/kit	R4717016
			Culti-Loops: 5 Loops/pack	R4607016

EE Broth Mossel

EE Broth Mossel is a modification of brilliant green bile broth, which contains purified ox bile (oxgall), in place of bile salts, and disodium phosphate to improve the buffering capacity of the medium and encourage early growth of indicator organisms. EE Broth Mossel is recommended by the United States Pharmacopeia (USP) for use in testing for the presence of bile-tolerant, Gram-negative bacteria by the American Public Health Association for use in the most probable number assay, and as an enrichment broth in straight enrichment procedures. EE Broth Mossel is formulated in conformance with harmonized United States Pharmacopeia (USP)/European Pharmacopeia (EP) guidelines.

Mode of Action

The undesired, accompanying bacterial flora is almost completely inhibited by brilliant green and ox bile. Dextrose favors the growth of all *Enterobacteriaceae*. The strong buffering capacity of the culture medium prevents the formed acid from killing the culture.

VRBG Agar (Violet Red Bile Glucose Agar)

Mossel et at. modified Violet Red Bile Agar by adding glucose to enable detection of nonlactose-fermenting *Enterobacteriaceae*. Further research demonstrated that lactose could be omitted, resulting in the formulation known as Violet Red Bile Glucose Agar (VRBGA). VRBGA is formulated in conformance with harmonized United States Pharmacopeia (USP)/European Pharmacopeia (EP) guidelines for use in testing for the presence of bile-tolerant, Gram-negative *bacilli*.

Classical Formula

Oxgall	20.0 g
Peptone	10.0 g
Disodium phosphate	8.0 g
Dextrose	5.0 g
Monopotassium phosphate	2.0 g
Brilliant green	15.0 mg
Demineralized water	1000.0 mL

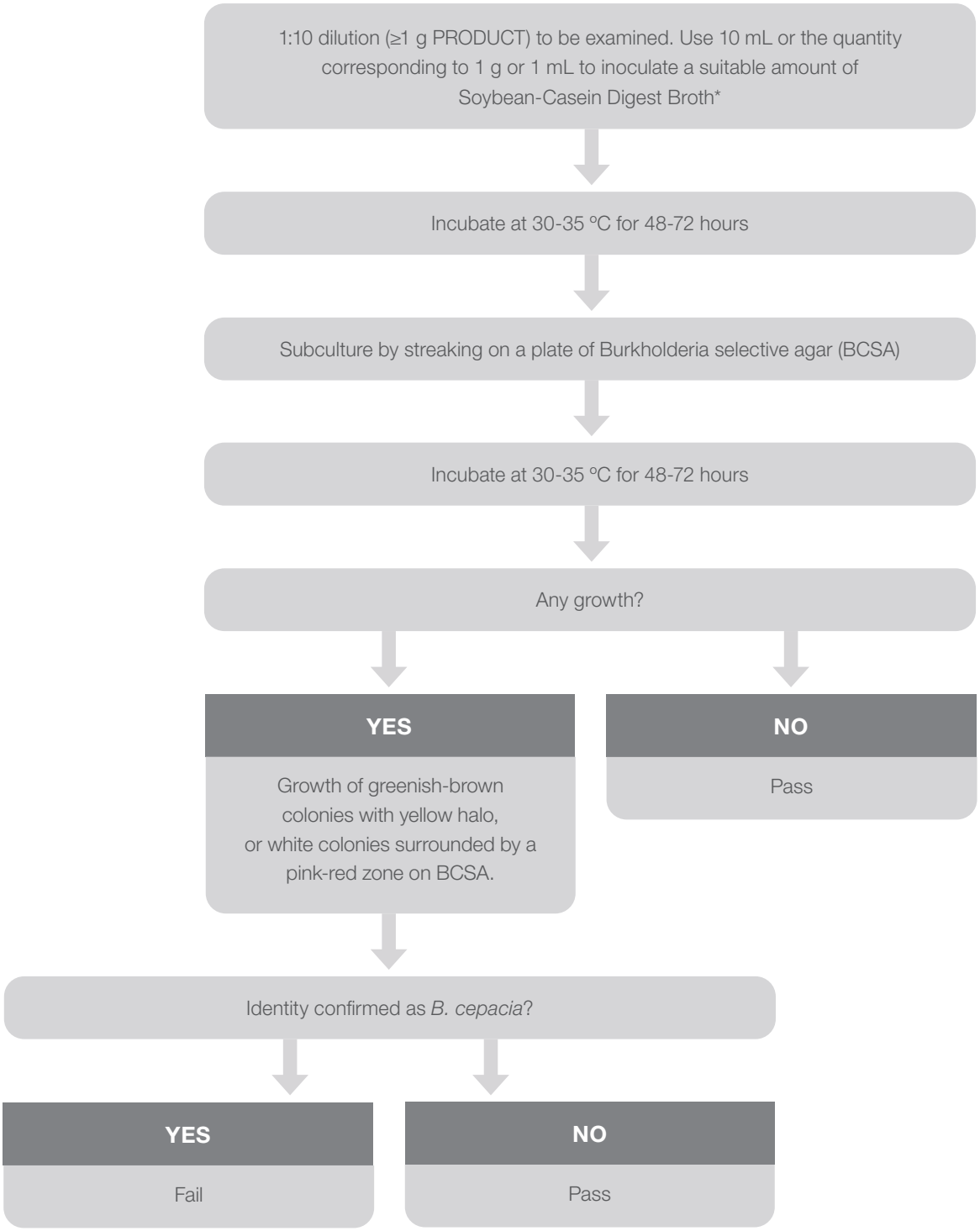
Mode of Action

Gelatin peptone supplies amino acids, peptides, and nitrogenous compounds essential for bacterial growth. Yeast extract provides essential B-complex vitamins and glucose is a carbon energy source. Sodium chloride maintains osmotic equilibrium. Bile salts and crystal violet are selective agents which inhibit the growth of Gram-positive organisms. Neutral red is an indicator of acid production. Gram-negative organisms which ferment glucose form colonies that are pink to red in color.

Classical Formula

Glucose	10.0 g
Bile salts	1.5 g
Gelatin peptone	7.0 g
Neutral red	30.0 g
Sodium chloride	5.0 g
Crystal violet	2.0 mg
Yeast extract	3.0 g
Agar	15.0 g
Demineralized water	1000.0 mL

Absence of *Burkholderia cepacia*



* Soybean-Casein Digest Broth = Tryptic Soy Broth (TSB)

Absence of *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.

Test	Product	Property	Format	Product code
Test for *B. 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 BSCA*	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 BSCA*	Quanti-Cult: 10 tests/kit	R4737016
			Quanti-Cult Plus: 100 tests/kit	R4717016
			Culti-Loops: 5 Loops/pack	R4607016

BCSA

Initially a new 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.

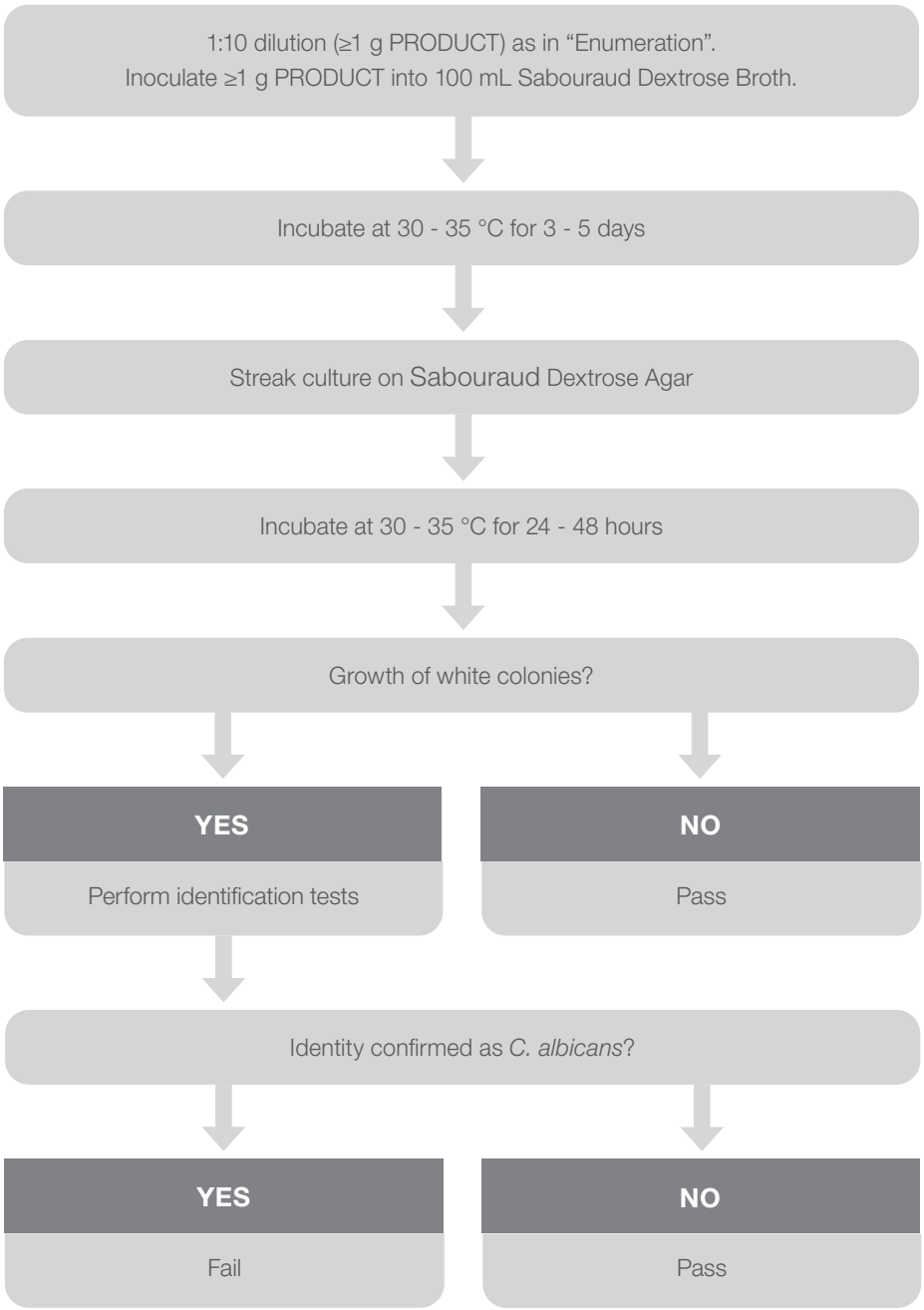
Mode of Action

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.

Classical Formula

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

Absence of *Candida albicans*



Absence of *Candida albicans*

Test	Product	Property	Format	Product code
Test for *C. 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
			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

More products available globally-see website or local representative

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.

Classical Formula

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.

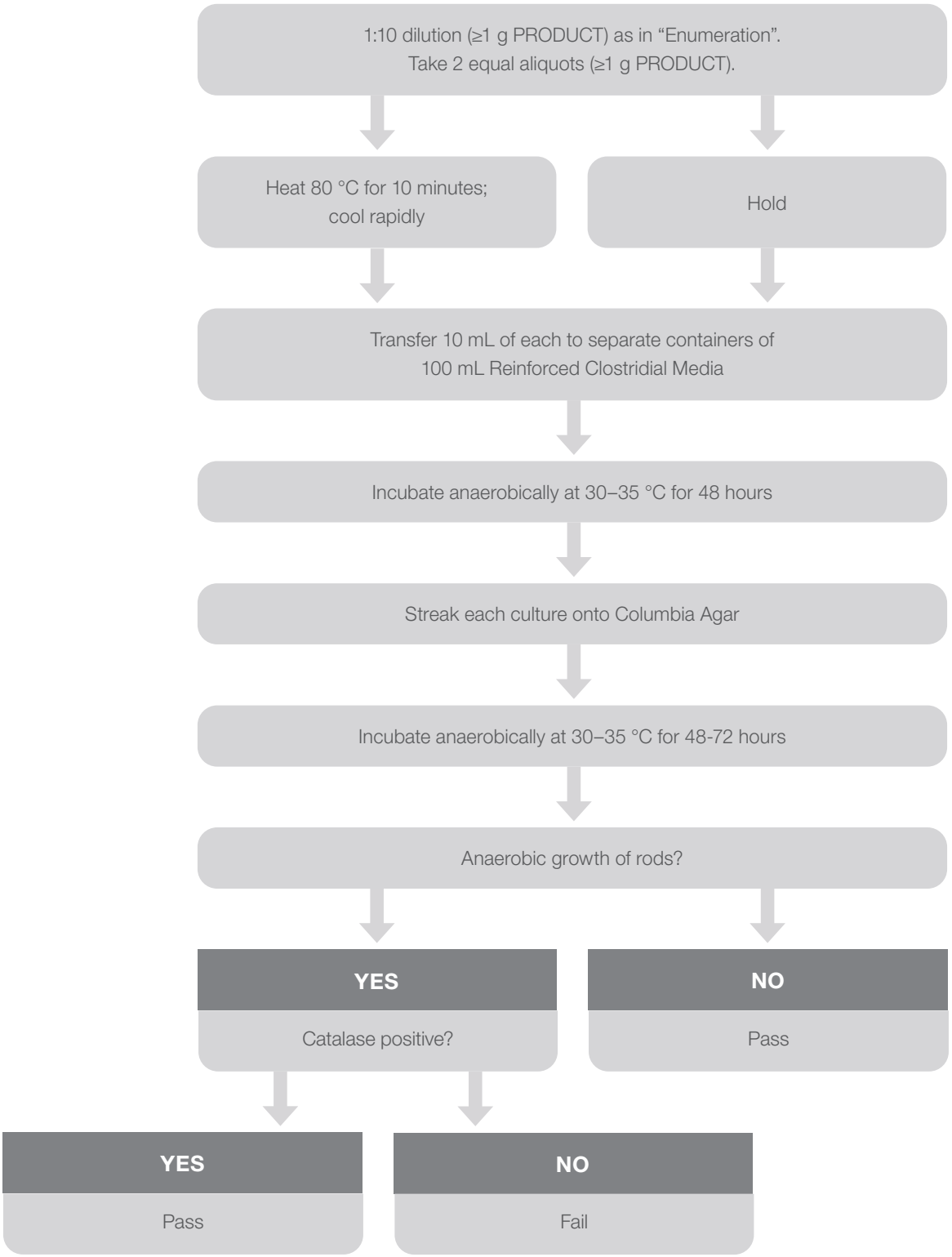
Mode of Action

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.

Classical Formula

Casein peptone	5.0 g
Dextrose	40.0 g
Meat peptone	5.0 g
Agar	15.0 g
Demineralized water	1000.0 mL

Absence of *Clostridium*



Absence of *Clostridium*

Test	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

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.

Classical Formula

Beef extract	10.0 g
Yeast extract	3.0 g
Peptone	10.0 g
Soluble starch	1.0 g
Dextrose	5.0 g
Cysteine hydrochloride	0.5 g
Sodium chloride	5.0 g
Agar	0.5 g
Sodium acetate	3.0 g
Demineralized water	1000.0 mL

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.

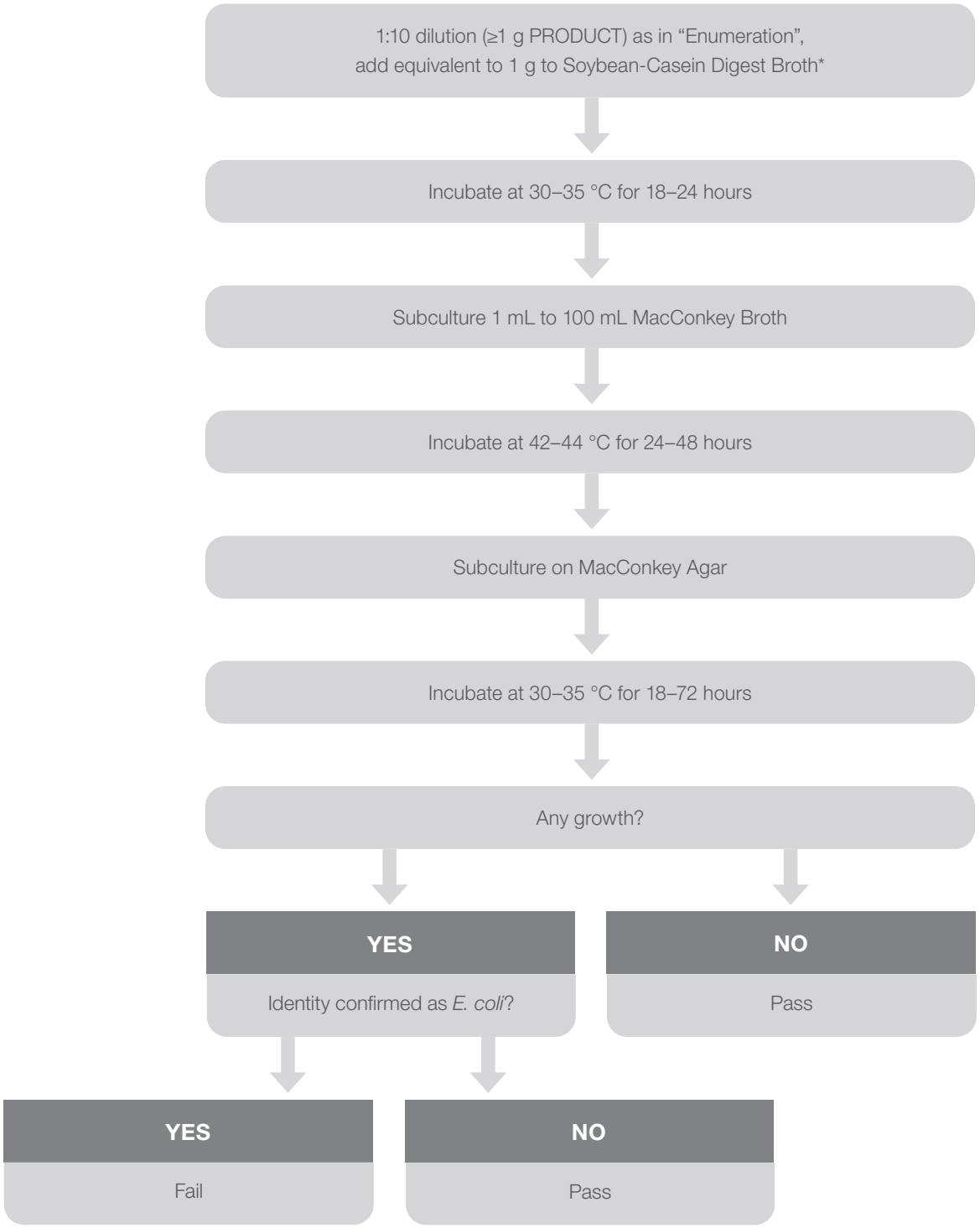
Mode of Action

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

Classical Formula

Pancreatic digest of casein	10.0 g
Heart pancreatic digest	3.0 g
Meat peptic digest	5.0 g
Corn starch	1.0 g
Sodium chloride	5.0 g
Agar	12.5 g
Yeast extract	5.0 g
Demineralized water	1000.0 mL

Absence of *Escherichia coli*



[a] Soybean-Casein Digest Broth = Tryptic Soy Broth (TSB)

Absence of *Escherichia coli*

This is a Gram-negative bacterium and is an indicator for fecal contamination. Such contamination could arise from poor hygiene of operators, contamination from feral animals, cats, birds or a low quality water supply amongst others. *Escherichia coli* is capable of causing diarrhea and sickness and some strains are capable of producing a potent verotoxin.

Test	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

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 in the medium serves to inhibit growth of Gram-positive organisms and replace sodium taurocholate used in the original formulation. MacConkey Broth is formulated in conformance with harmonized United States Pharmacopeia (USP)/European Pharmacopeia (EP) guidelines.

Mode of Action

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

Classical Formula

Gelatin peptone	20.0 g
Oxgall	5.0 g
Lactose	10.0 g
Bromcresol purple	0.01 g
Demineralized water	1000.0 mL

MacConkey Agar

In 1900, MacConkey first described a neutral red bile salt medium for cultivation and identification of enteric organisms. A detailed description of the selective and differential properties of the medium was published in 1905. Over the years, MacConkey's original formula has been 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 resulted has demonstrated improved inhibition of swarming by *Proteus* spp.

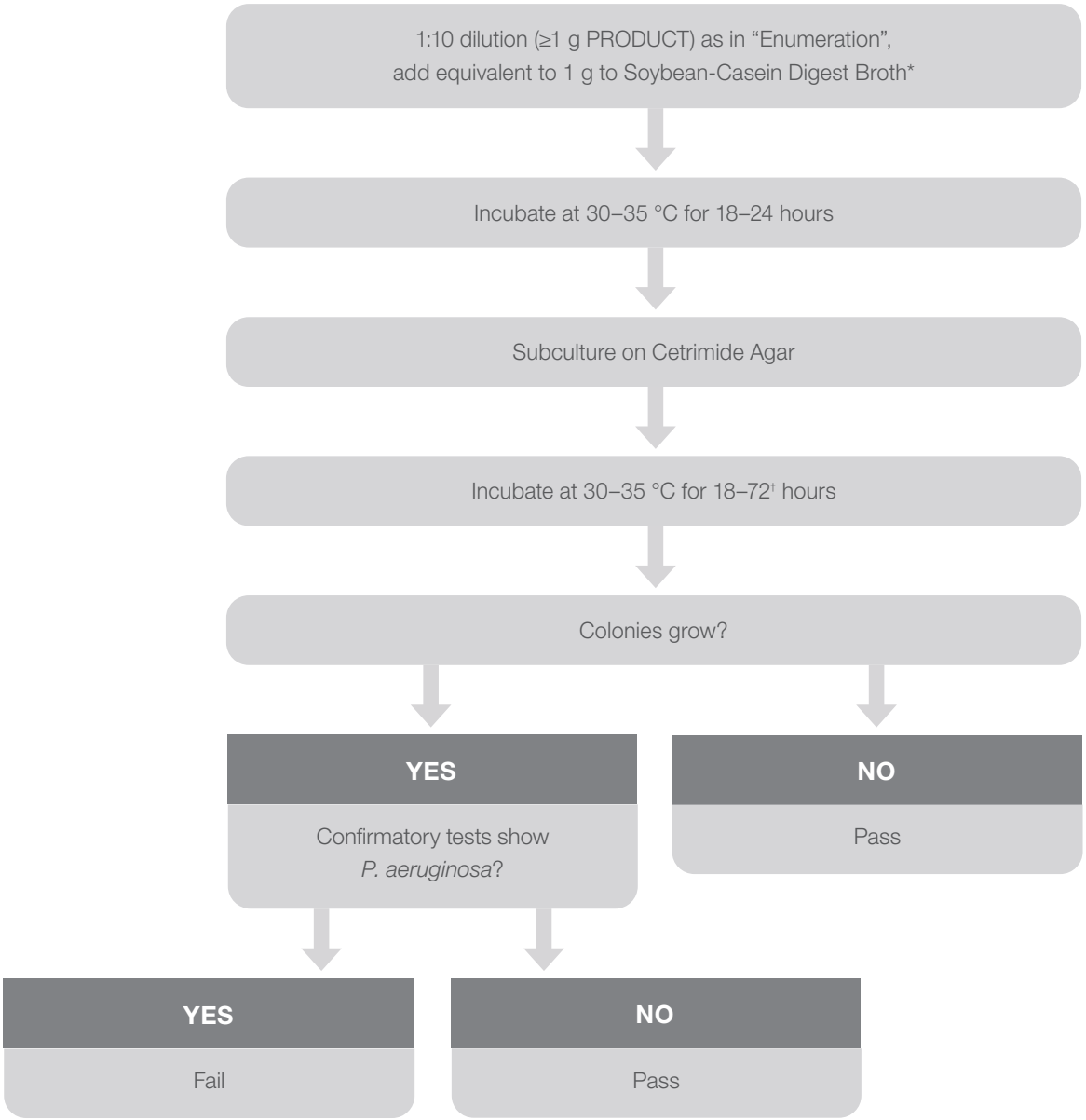
Mode of Action

Peptones provide nitrogenous nutrients and amino acids necessary for bacterial growth. Lactose is a carbon source for energy. Sodium chloride supplies essential electrolytes and maintains osmotic equilibrium. Crystal violet and bile salts are selective agents which inhibit most Gram-positive organisms. Differentiation of Gram-negative bacilli is accomplished by addition of lactose and neutral red which is an indicator.

Classical Formula

Gelatin peptone	17.0 g
Meat peptone	1.5 g
Lactose	10.0 g
Neutral red	30.0 g
Sodium chloride	5.0 g
Crystal violet	1.0 g
Bile salts	1.5 g
Agar	13.5 g
Casein peptone	1.5 g
Demineralized water	1000.0 mL

Absence of *Pseudomonas aeruginosa*



* Soybean-Casein Digest Broth = Tryptic Soy Broth (TSB)

† Incubation conditions not defi ned in USP. JP instructs incubation at 30–35 °C for 24–48 hours for all plate incubations.

Absence of *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.

Test	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

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.

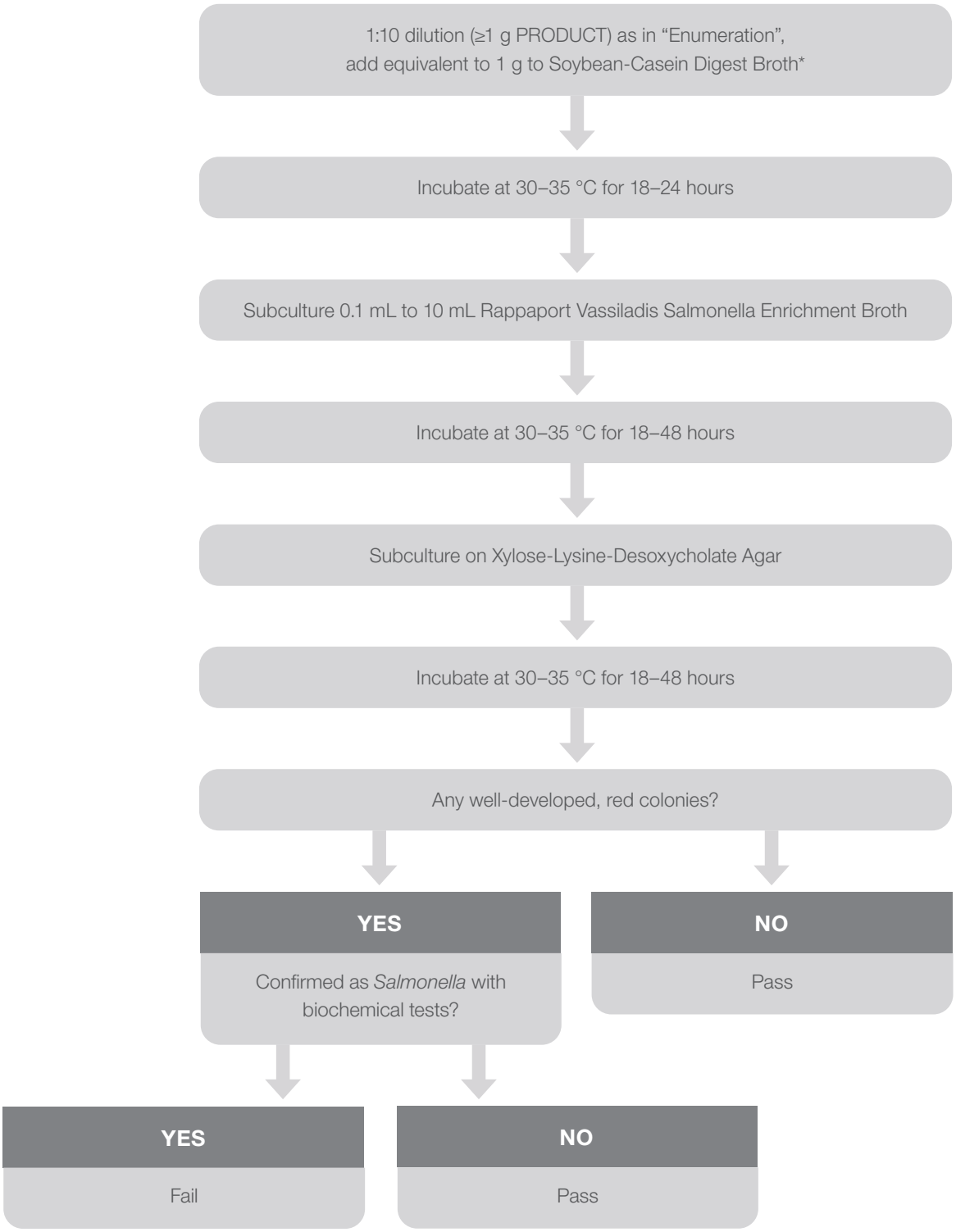
Classical Formula (g / litre)

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

Also to be added:

Glycerol	10.0 mL

Absence of *Salmonella* Typhimurium



[A] Soybean-Casein Digest Broth = Tryptic Soy Broth (TSB)

Absence of *Salmonella* Typhimurium

Salmonella enterica, subspecies *enterica*, serovar Typhimurium is of fecal origin and can cause severe diarrhea and sickness with the resultant dehydration potentially fatal in children and the elderly who are at greatest risk.

Test	Product	Property	Format	Product code
Test for *Salmonella enterica* spp. 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

RVS (Rappaport-Vassiliadis Salmonella) Enrichment Broth MLT

Rappaport et al. formulated an enrichment medium for selective recovery of *Salmonella* spp. Vassiliadis et al. modified the formula by reducing the concentration of malachite green and magnesium chloride, creating Rappaport-Vassiliadis (RV) Broth. RVS Enrichment Broth MLT is formulated in conformance with harmonized United States Pharmacopeia (USP)/ European Pharmacopeia (EP) guidelines for use in testing for the presence of *Salmonella* spp.

Mode of Action

Soya Peptone is the source of carbon and nitrogen, magnesium chloride raises the osmotic pressure, and potassium dihydrogen phosphate acts as a buffer. Malachite green is a selective agent which is inhibitory to organisms other than *Salmonella* spp. The low pH of RVS Enrichment Broth MLT combined with the presence of malachite green and magnesium chloride creates an environment which facilitates selective recovery of *Salmonella* spp. from contaminated sources. Lowering the pH to 5.2 increases selectivity.

Classical Formula

Magnesium chloride	13.6 g
Potassium dihydrogen phosphate	0.6 g
Sodium chloride	5.0 g
Dipotassium phosphate	0.4 g
Soya peptone	4.5 g
Malachite green	36.0 g
Demineralized water	1000.0 mL

XLD Agar (Xylose Lysine Desoxycholate)

This medium was developed by Taylor for selective isolation and differentiation of enteric pathogens, especially Shigella spp.
XLD Agar has since been found to be a satisfactory medium for the recovery of *Salmonella* spp. from clinical specimens.

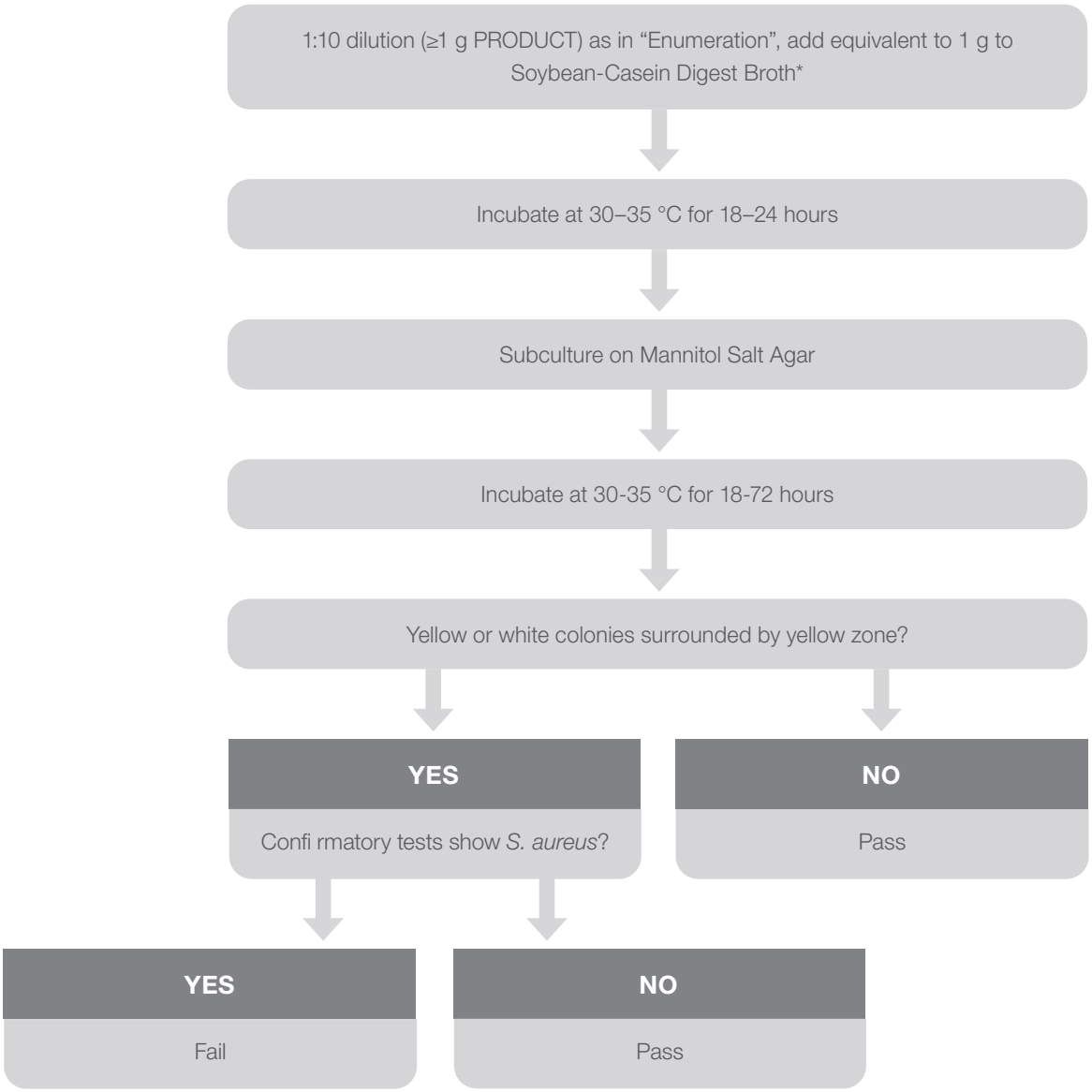
Mode of Action

Xylose is rapidly fermented by most enteric Gram-negative bacilli other than *Shigella* spp., producing red colonies. Lysine provides for differentiation of *Salmonella* spp. from nonpathogenic enteric Gram-negative bacilli. *Salmonella* produces lysine decarboxylase which causes the pH to revert to alkaline after xylose is fermented, producing red colonies. *Sodium thiosulfate*, a sulfur source, and ferric ammonium citrate, an indicator, are added to enable organisms which form hydrogen sulfide (H,S) to produce black-centered colonies under alkaline conditions. Such organisms include *Salmonella* spp. Organisms which ferment xylose, lactose, or sucrose and are lysine negative cause an acid pH and produce yellow colonies. Desoxycholate is a selective agent which inhibits Gram-positive organisms.

Classical Formula

Lactose	7.5 g
Yeast extract	3.0 g
Sucrose	7.5 g
Sodium desoxycholate	2.5 g
Sodium thiosulfate	6.8 g
Ferric ammonium citrate	0.8 g
L-lysine	5.0 g
Phenol red	0.08 g
Sodium chloride	5.0 g
Agar	13.5 g
Xylose	3.5 g
Demineralized water	1000.0 mL

Absence of *Staphylococcus aureus*



* Soybean-Casein Digest Broth = Tryptic Soy Broth (TSB)

† Incubation conditions not defined in USP. JP instructs incubation at 30–35 °C for 24–48 hours for all plate incubations.

Absence of *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.

Test	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

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.

Mode of Action

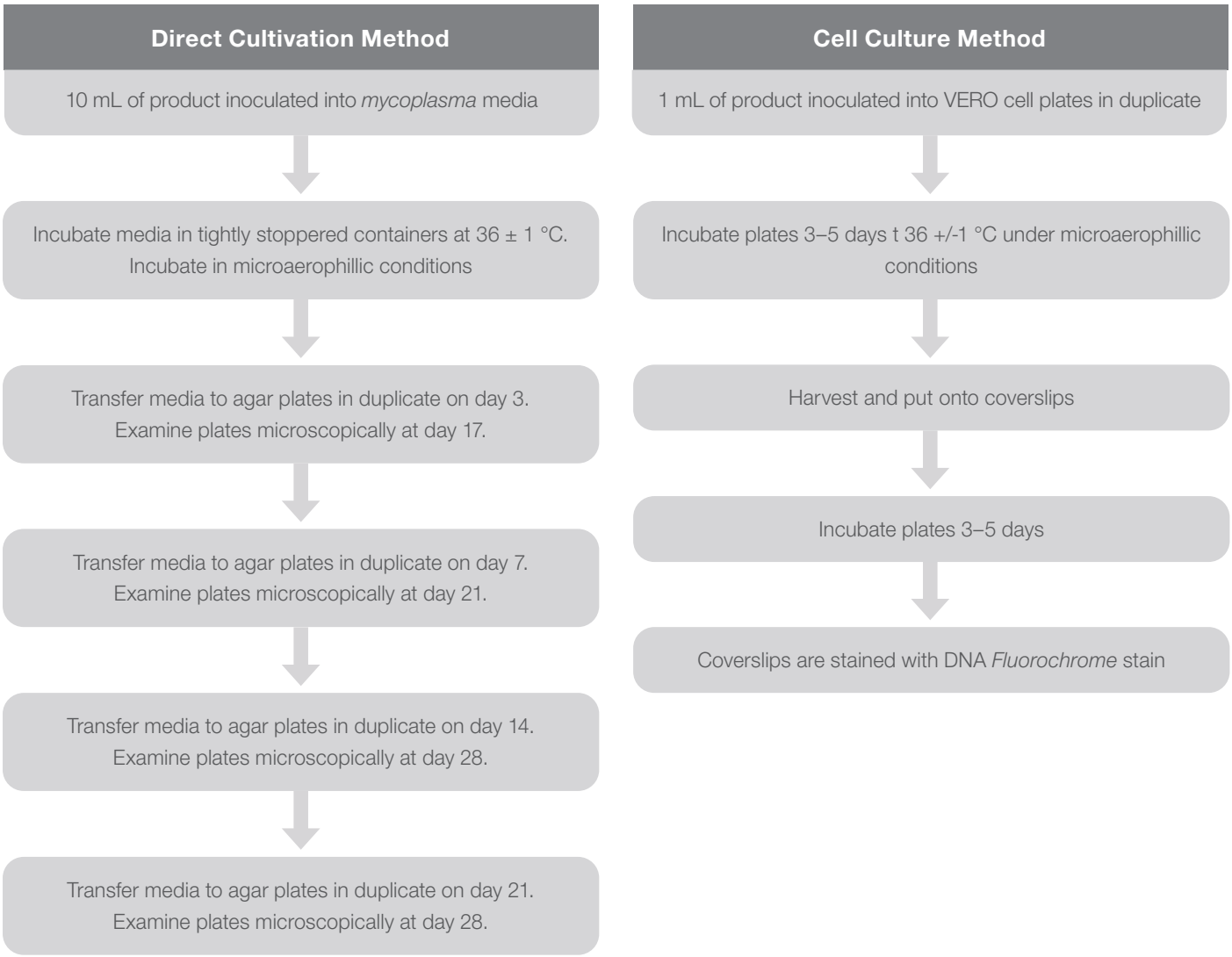
Casein and meat peptones supply nitrogen, amino acids, and peptides necessary for bacterial growth. Sodium chloride in a concentration of 7.5% is a selective agent which inhibits many bacteria other than staphylococci. Phenol red is a pH indicator which causes a color change in the medium from red-orange 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.

Classical Formula

Sodium chloride	75.0 g
Beef extract	1.0 g
D-mannitol	10.0 g
Phenol red	25.0 mg
Casein peptone	5.0 g
Agar	15.0 g
Meat peptone	5.0 g
Demineralized water	1000.0 mL

Routine *Mycoplasma* Testing

Two testing methods:



Control plates utilizing *Mycoplasma orale* and *Mycoplasma pneumoniae* are prepared alongside the test products.

The test passes if *Mycoplasma* is not detected using the direct and cell culture methods. The controls must exhibit growth when inoculated with ≤100 CFU per organism. All negative controls must be free of *Mycoplasma* contamination.

Routine *Mycoplasma* Testing

Mycoplasmas are a significant problem for the biopharmaceutical industry, because they are a very common cause of contamination in cell cultures used in research laboratories and in industrial processes. The bacteria thrive in cell culture media and can reach very high numbers without causing visible changes in the culture, or affecting its viability, and so remain undetected.

The purpose of the test is to determine the presence or absence of mycoplasmal contaminants in the cell cultures samples. The FDA requires that both assays are performed in order to ensure a high degree of certainty in confirming the presence or absence of *Mycoplasma* contamination. The testing challenges consist of the two methods on the previous page.

Test	Medium	Format	Product code
PPLO Broth	w/horse serum, yeast extract. For cultivation of *Mycoplasma* spp.	15 x 103 mm tube, 5 mL 20/pk	R20360
PPLO Agar	w/horse serum, amphotericin B, penicillin, thallium acetate. For selective isolation of *Mycoplasma* spp.	15 x 60 mm plate 10/pk	R20260
SP4 Glucose Broth	w/thallium acetate, penicillin. For isolation of *Mycoplasma* spp. Penicillin inhibits growth of Gram-positive bacteria. REF R112585 not intended for IVD use.	15 x 103 mm tube, 5 mL 20/pk	R20376
SP4 Glucose Agar	w/thallium acetate, penicillin. For isolation of *Mycoplasma* spp. Penicillin inhibits growth of Gram-positive bacteria.	15 x 60 mm plate 10/pk	R20276

Table 1. Methodological differences between the new USP chapter <63>, EP 2.6.7, and the 1993 PTC

Requirements	USP<63>	EP 2.6.7	1993 PTC
Nutritive properties	The solid medium complies with the test if a count within 0.5-log unit range of the inoculate amount is found for each test microorganism	The solid medium complies with the test if growth obtained does not for differ by a factor >5 from value calculated with respect to the inoculum	Not addressed; reference made to the 21 CFR 610.30
Inhibitory substances	If plates inoculated with the test article/material are not within a 0.5-log unit range of the number of colonies of those without, inhibitory substances are present	If plates inoculated with the product to be examined have fewer than 1/5 of the number or colonies of those inoculated without the product, inhibitory substances are present	Not addressed
Quality Control strain organisms	At least two *mycoplasma* species should be included as positive controls (one dextrose fermenter and one arginine hydrolyzer)	At lease one of the species listed will be included as a positive control	At least two *Mycoplasma* species should be included as positive controls (one dextrose fermenter and one arginine hydrolyzer)
Quality Control strain organisms storage	Stored frozen (at -20 °C or lower) or freeze-dried	Stored frozen or freeze-dried	Not addressed
Number of subcultures	Positive control organisms to be used not more than 15 passages from isolation	Positive control organisms to be used not more than 15 subcultures from isolation	Positive control organisms to be used not more than 15 passages from isolation
Incubation conditions	Tightly stoppered containers at 36 ± 1 °C	Tightly stoppered containers at 35–38 °C	36 ± 1 °C