1.0 Purpose

The purpose of this procedure is to outline the growth promotion and sterility testing of vendor received, ready-made and dehydrated media.

2.0 Scope

This procedure is used to determine the effectiveness of media used for Microbiological testing at Ion Labs and to ensure all media prepared/purchased is suitable for use in the Micro Lab.

3.0 Responsibility

3.1 It is the responsibility of QC Laboratory Analysts to ensure all procedures and specifications are met.

3.2 It is the responsibility of QC Laboratory Management to implement this procedure and to ensure that the procedure is being followed.

3.3 It is the responsibility of QC Laboratory Management to keep current this procedure and to oversee validations and recovery studies.

4.0 Definitions

4.1 **NLT** – No Less Than

4.2 **LT** – Less Than

4.3 **CFU** – Colony Forming Units

4.4 **QC** – Quality Control

4.5 **Dehydrated Media** – Dry media consisting of best ingredients like, peptones, vegetable extracts, animal tissue, sodium salts, inhibitory agents, agar content and carbohydrate sources for the media preparation and growth of microorganisms.

4.6 **Ready-Made Media** – Media which has been prepared and is ready to use upon receipt.

4.7 **TSB** – Tryptic Soy Broth

4.8 **RVS** – Rappaport-Vassiliadis Soya Broth

4.9 **EE** – Enterobacteriaceae Enrichment Broth

4.10 **MRS** – De Man, Rogosa and Sharpe Agar

4.11 **TOS** – Transgalactosylation Oligosaccharide Agar

4.12 **SDA** – Sabouraud Dextrose Agar

4.13 **RYM** – Rapid Yeast and Mold Petrifilm

4.14 **TSA** – Tryptic Soy Agar

4.15 **LB Broth** – Luria-Bertoni Broth

4.16 **LB Agar** – Luria-Bertoni Agar

4.17 **LB Soft Agar** – Luria-Bertoni Soft Agar

4.18 **AC** – Aerobic Count Petrifilm

4.19 **VRBG** – Violet Red Bile Glucose Agar

4.20 **EC** – E. coli/Coliform Count Petrifilm

4.21 **MSA** – Mannitol Salt Agar

4.22 **STX** – Staph Express Count Petrifilm

4.23 **CET** – Cetrimide Agar

4.24 **XLD** – Xylose Lysine Deoxycholate Agar

4.25 **EB** – Enterobacteriaceae Count Petrifilm

4.26 **BCSA** – Burkholderia cepacia Agar

4.27 **COA** – Certificate of Analysis

4.28 **DRBC** – Dichloran Rose-Bengal Chloramphenicol Agar

4.29 **BA** – Blood Agar

4.30 **MAC** – MacConkey

5.0 References

5.1 D-715, SOP, Microbial Limit Testing Using 3M™ Petrifilm™ System

5.2 D-715.0, SOP, Microbial Limit Testing Using Agar Plates

5.3 D-716, SOP, Enumeration of Raw Materials for Probiotic Supplements

5.4 D-126, SOP, Non-Conforming Results in the QC Laboratory

5.5 D-113-F1, Form, Microbial Media Validation Testing Log

5.6 D-706, SOP, Use and calibration of pH Meters

5.7 C-502, SOP, Record Storage, Retention, and Destruction

5.8 A-106, SOP, Documentation Guidelines for cGMP Records

5.9 C-501, SOP, Document Control Procedure

5.10 21 CFR Part 110 and 111

5.11 USP Chapter<2021>, <2022>, <71> and <1116>

6.0 Required Supplies, Media, and Equipment

6.1 Ready-made and in-house made Media

6.2 Control Organisms include but are not limited to:

6.2.1 Aerobic Bacteria

6.2.1.1 *Staphylococcus aureus*, ATCC 6538

6.2.1.2 *Bacillus subtilis (spizenzi),* ATCC 6633

6.2.1.3 *Pseudomonas paraeruginosa,* ATCC 9027

6.2.1.4 *Burkholderia cepacia,* ATCC 25416

6.2.1.5 *Salmonella enterica,* ATCC 14028

6.2.1.6 *Escherichia coli,* ATCC 8739

6.2.1.7 *Listeria monocytogenes,* ATCC 13932

6.2.2 Fungi

6.2.2.1 *Candida albicans*, ATCC 10231

6.2.2.2 *Aspergillus niger*, ATCC 16404

6.2.3 Probiotic

6.2.3.1 *Bifidobacterium spp.* (e.g. *B. longum, B. bifidum, B. breve*)

6.2.3.2 *Lactobacillus spp.* (e.g. *L. rhamnosus, L. acidophilus*)

6.2.3.3 *Bacillus coagulans*, ATCC 7050

6.3 Adjustable Incubators

6.3.1 20°C - 25°C

6.3.2 30°C - 35°C

6.3.3 35°C - 39°C

6.4 Adjustable Water Bath

6.4.1 42°C - 44°C

6.5 Biological Safety Cabinet

6.6 Sterile Pre-saturated 70% Isopropyl Alcohol (IPA) Wipes

6.7 Sterile 70% Isopropyl Alcohol (IPA)

6.8 Cleanroom Indelible Marker

6.9 100 µL pipettes

6.10 Sterile Pipette tips

6.11 Sterile Gloves

7.0 Procedure

7.1 Growth Promotion Organism Preparation

7.1.1 Commercially prepared challenge organisms may be used. The ATCC control strains disclosed in COA (Certificate of Analysis) from media vendor are suitable for use, in addition to the control organisms listed in this SOP.

7.1.2 Prepare the challenge organisms per manufacturer instruction. Use the

preparation within 8 hours.

7.1.3 Pull challenge organism into a 1 cc sterile syringe/needle or use a pipette to aspirate the inoculum into a sterile pipette tip to inoculate plate or liquid media. This is used to inoculate test media and control media.

Note: The quantity of countable microbes must be between 10-100CFU per plate.

7.2 Media Preparation

7.2.1 For dehydrated media, refer to the instructions on the bottle label and sterilize the media prior to use in accordance with SOP D-824: Operation and Cleaning of the Tuttnauer EZ10 Autoclave.

7.3 Growth Promotion Testing of Media

7.3.1 Each preparation of prepared media and each lot of purchased media is required to be challenged for growth promotion.

7.3.2 Obtain two test media for each organism to be tested. Label each appropriately with the organism name, date and initials.

7.3.3 Obtain two control media (TSA or previous lot of the media type to test), which have previously been tested and passed growth promotion. These are the positive controls. Alternatively, AC petrifilm can be use as control media for petrifilm validation.

7.3.4 Inoculate the test media and control media by placing an inoculum containing ≤100 CFU, based on CFU/0.1mL on organism's package label, in/on each test item. For petrifilm, prepare 1ml of Butterfields Buffer or Peptone Salt diluent containing 100ul of challenge organism, per film to inoculate.

7.3.5 Use a sterile inoculating needle, loop, or plate spreader to spread the organism across any test plates or control plates to evenly spread the organism. Liquid

media does not require this step.

7.3.6 Obtain at least two test media to be incubated as negative control of test lot. Petrifilm negative controls are inoculated with sterile diluent only.

7.3.7 Incubate the test and control media at the appropriate temperature for the media and/or challenge organism, see Table 1.

7.3.8 Record the organisms used on D-113-F1 Microbial Media Validation Testing Log.

7.3.9 After Incubation

7.3.9.1 If the test media is qualitative media, examine the test media and record growth or no growth on D-113-F1 Microbial Media Validation Testing Log. Qualitative media include broth or other liquid media, as well as selective and differential media.

7.3.9.2 If the test media is quantitative media, examine the test media and record the number of CFU per plate on D-113-F1 Microbial Media Validation Testing Log.

7.3.9.3 Examine the control media; record the number of CFU/plate on D-113-F1 Microbial Media Validation Testing Log.

7.3.9.4 Control media must have 10-100 CFU per plate per organism for the test to be valid.

7.3.9.5 Negative Control media must contain 0 CFU or no growth in order for the tests to be valid.

7.3.10 Calculate the percent recovery for quantitative media. (i.e., test count average divided by control count average X 100).

7.3.10.1 Percent recovery between the test plates and control plates must be 50-

200% for plates to be considered acceptable for use.

7.3.11 If any organism fails to meet 50-200% recovery for test plates, retest the test plates in quadruplicate. The control plates will be performed in duplicate.

7.3.11.1 The retest may be performed by the same or different lab personnel.

7.3.11.2 If the media fails a second time, the media is rejected for use in the laboratory.

7.3.12 Download and store manufacturer COA in digital folder labelled "COA Media Validation" for review.

7.4 Sterility

7.4.1 Incubate the negative control for sterility check at specified temperature for time specified as per Table 1.

7.4.2 There should not be any microbial growth recovered on the negative control sterility media after incubation period.

7.4.3 Document results observed on Form D-113-F1 Microbial Media Validation Log.

7.5 pH measurement

7.5.1 Allow test media to be equilibrated at room temperature before pH measurement. Petrifilm does not require pH readings.

7.5.2 The pH meter must be calibrated before use. Refer SOP D-706 Use and calibration of pH Meters.

7.5.3 Acceptance criteria for pH readings is defined per each culture media, see Table 1.

7.5.4 If observed pH values do not meet these criteria, refer to COA vendor for acceptable results. Document results observed on Form D-113-F1.

Table 1- Media Sterility and Growth Promotion Testing, Incubation, and pH

Media	Growth Promotion Organism	Temperature Requirement	Incubation Time	pH
Chocolate Agar	Bacillus subtilis ATCC 6633	30-35°C	18 - 24 Hours Sterility incubation is NLT 18 Hours	7.2 ± 0.2
BA	Staphylococcus aureus ATCC 6538	30-35°C	18 - 24 Hours Sterility incubation is NLT 18 Hours	7.3 ± 0.2
TSB	Escherichia coli ATCC 8739 Staphylococcus aureus ATCC 6538 Pseudomonas aeruginosa ATCC 9027 Salmonella enterica ATCC 14028 Burkholderia cepacia ATCC 25416	30-35°C	18 - 24 Hours Sterility incubation is NLT 3 days	7.3 ± 0.2
TSA or AC Petrifilm	Staphylococcus aureus ATCC 6538 Pseudomonas aeruginosa ATCC 9027 Burkholderia cepacia ATCC 25416 Bacillus subtilis ATCC 6633	30-35°C	18 - 24 Hours Sterility incubation is NLT 3 days	7.3 ± 0.2 (TSA only)
MAC Agar or EC Petrifilm	Escherichia coli ATCC 8739	30-35°C	18 - 24 Hours Sterility incubation is NLT 18 hours	7.1 ± 0.2 (Mac only)
VRB	Escherichia coli ATCC 8739	30-35°C	18 - 24 Hours Sterility incubation is NLT 18 Hours	7.2 ± 0.2
MAC Broth	Escherichia coli ATCC 8739	42-44°C	18 - 24 Hours Sterility incubation is NLT 18 Hours	7.1 ± 0.2
MSA or STX Petrifilm	Staphylococcus aureus ATCC 6538	30-35°C	18 - 24 Hours Sterility incubation is NLT 18 Hours	7.4 ± 0.2 (MSA only)
XLD or EB Petrifilm	Salmonella enterica ATCC 14028	30-35°C	18 - 24 Hours Sterility incubation is NLT 18 Hours	7.4 ± 0.2 (XLD only)
EE Broth Mossel	Salmonella enterica ATCC 14028	30-35°C	18 - 24 Hours Sterility incubation is NLT 18 Hours	7.2 ± 0.2
RVS Broth	Salmonella enterica ATCC 14028	30-35°C	18 - 24 Hours Sterility incubation is NLT 18 Hours	5.1 ± 0.2
VRBG or EB Petrifilm	Salmonella enterica ATCC 14028	30-35°C	18 - 24 Hours Sterility incubation is NLT 18 Hours	7.4 ± 0.2 (VRBG only)
BCSA Agar	Burkholderia cepacia ATCC 25416	35-39°C	24-48 hours Sterility incubation is NLT 2 days	7.0 ± 0.2
LB Broth	E. Coli ATCC 8739	35-39°C	18-24 hours Sterility incubation is NLT 18-24 Hours	7.0 ± 0.2
LB Agar, LB Soft Agar	E. Coli ATCC 8739	35-39°C	18-24 hours Sterility incubation is NLT 18-24 Hours	7.0 ± 0.2
CET Agar	Pseudomonas paraeruginosa ATCC 9027	30-35°C	18 - 24 Hours Sterility incubation is NLT 18 Hours	7.2 ± 0.2
HARDYCHROM ECC	E. coli ATCC 8739	35-37°C	18 - 24 Hours Sterility incubation is NLT 18 Hours	7.0 +/- 0.3
HARDYCHROM Salmonella	Salmonella enterica ATCC 14028	35-37°C	24 Hours Sterility incubation is NLT 24 Hours	7.2 +/- 0.2
HARDYCHROM Listeria	Listeria monocytogenes ATCC 13932	35-37°C	24 Hours Sterility incubation is NLT 24 Hours	7.2 +/- 0.2
HARDYCHROM Staph aureus	Staphylococcus aureus ATCC 6538	35-37°C	20 - 28 hour Sterility incubation is NLT 20 Hours	7.05 +/- 0.25
HARDYCHROM Candida	Candida albicans ATCC 10231	30-35°C	48 Hours Sterility incubation is NLT 48 Hours	6.1 +/- 0.2
SDA, DRBC and RYM Petrifilm	Candida albicans ATCC 10231 Aspergillus niger ATCC 16404	20-25°C	3 - 5 Days Sterility incubation is NLT 3 days	5.6 ± 0.2 (SDA and DRBC only)
MRS Agar	Lactobacillus spp.*	35-39°C	2 - 3 Days Sterility incubation is NLT 2 days	5.5 ± 0.2
TOS Agar	Bifidobacterium spp.*	35-39°C	3 Days Sterility incubation is NLT 2 days	6.3 ± 0.2
OGYE Agar	Bacillus coagulans*	35-39°C	2 - 3 Days Sterility incubation is NLT 2 days	7.0 ± 0.2

* Incubate anaerobically

8.0 Use and Disposition

8.1 Media (prepared or purchased) once made or received will be labeled with a quarantine sticker until testing is started. No media should be used in this state.

8.2 Media (prepared or purchased) that does not pass pH, growth promotion, or sterility will not be used for Microbial Testing and immediately discarded or returned to vendor.

8.3 Media (prepared or purchased) that meets all acceptance requirements will be tagged with a sticker indicating QC approval. Only use of approved media lots is allowed.

9.0 Documentation Requirements

9.1 A PQV check must be performed for each completed page of form D-113-F1 Microbiological Media Validation Log as outlined in SOP A-106 Documentation Guidelines for cGMP Records.

9.2 All documentation will be distributed and maintained as outlined in SOP C-501 Document Control and SOP C-502 Record Storage, Retention, and Destruction.

10.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	04/14/20	New procedure.	N/A	L. McWade
1	05/19/21	Added specific amount of organism to add under growth promotion section.	CC-21-0191	G. Shaw
2	06/06/22	Added BCSA Media to Table 1 and 2 and corrected a couple agars results.	CC-22-0260	G. Shaw
3	10/05/22	Updated SOP to follow current guidelines.	CC-22-0402	G. Shaw
4	05/11/23	Added requirement to challenge all batches of prepared media for growth promotion. Other minor clarifications. Updated logo.	CC-23-0242	J. Sassman
5	07/12/23	Adjusted incubation times and sterility incubation times. Added numerous organisms that were missing. Adjusted form for more clear recordings and added pH space.	CC-23-0337	G. Shaw
6	06/11/24	Updated SOP to follow current practices.	CC-24-0269	A. Perez
7	12/05/25	Updated SOP to follow current practices.	CC-25-0487	V. Ortiz