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1.0 Purpose

This procedure defines the testing of various types of culture media to determine their sterility assurance level and growth promotion capabilities.

2.0 Scope

This SOP applies to the determination of sterility and growth capabilities of microbial growth media used to support manufacturing, validation, and Process Analytics/Quality Control (PA/QC) operations at the Biopharmaceutical Development Program (BDP).

3.0 Authority and Responsibility

3.1 The Director of Process Analytics/Quality Control (PA/QC) has the authority to define this procedure.

3.2 PA/QC is responsible for training laboratory personnel and documenting this training to Biopharmaceutical Quality Assurance (BQA).

3.3 PA/QC personnel are responsible for performance of this procedure.

3.4 PA/QC is responsible for reviewing the data and documentation of the results of this procedure.

3.5 BQA is responsible for quality oversight of this operation.

4.0 Materials

4.1 Microbial growth media to be tested.

4.2 Biological Safety Cabinet (BSC).

4.3 Incubator, 20-25°C.

4.4 Incubator, 30-35°C.

4.5 Incubator, 35-37°C.

4.6 Sterile PBS, BDP PN 30007.

4.7 Rotating Stand.

4.8 Vortex Mixer.

4.9 Septihol, BDP PN 30129 (sterile 70% Isopropyl alcohol).

4.10 Accessory Growth Promotion Funnel, BDP PN 31364

4.11 Foam rack.

4.12 Sterile disposable spreaders, BDP PN 20670.

4.13 Sterile gloves.

4.14 Sterile sleeves.

4.15 Challenge organisms:

- *Aspergillus brasil (A.brasil)* American type culture collection (ATCC) No. 16404, BDP PN 30812
- *Bacillus subtilis (B. subtilis)* ATCC No. 6633, BDP PN 30817
- BL21-AI, BDP PN 10401, or BDP approved equivalent
- BL21(λDE3)pLysS, BDP PN 50196 or BDP approved equivalent
- *Candida albicans (C. albicans)* ATCC No. 10231, BDP PN 30818
- DH5α, BDP PN 10306, or BDP approved equivalent
- DH5α pING-mTYRP2, BDP PN 10392 or BDP approved equivalent
- DH10B, BDP PN 50197, or BDP approved equivalent
- *Escherichia coli (E. coli)* ATCC No. 8739, BDP PN 30820
- *Pseudomonas aeruginosa (P. aeruginosa)* ATCC No. 9027, BDP PN 30821
- *Staphylococcus aureus (S. aureus)* ATCC No. 6538, BDP PN 30822
- *P. pastoris* Accessioning Bank, BDP PN 50431 (or equivalent)
- Quanti-Cult Plus Nutritional Adequacy Testing QC Set, BDP PN 31383

5.0 Procedure

Observe practices pertaining to sterile technique when performing this procedure. Use the appropriate personal protective equipment (PPE) including, but not limited to, safety glasses, laboratory coat, gloves, and sleeves.

NOTE: All plates and tubes shall be labeled with the QC Test Request Number, Date, Initials, and challenge organism (if applicable) at a minimum. Reference Attachment 1 for media types and challenge organisms. All plates will be labeled on the bottom of the plate.

5.1 Principles

5.1.1 Test 10% or a maximum of 10 samples per lot and/or each shipment for sterility.

5.1.2 Test each medium per lot and/or each shipment for growth promotion.

5.1.3 Use culture suspensions that will recover growth of <100 cfu/mL.

5.1.4 Perform all testing within the BSC.

5.2 Sterility

5.2.1 Obtain the media to be tested.

5.2.2 Record the following information on Form 22712-01, Media Qualification Testing.

5.2.2.1 Medium type

5.2.2.2 BDP Lot number

5.2.2.3 Expiration date

5.2.2.4 Equipment information

5.2.3 Label the samples.

5.2.4 Incubate the samples at the temperature and duration specified for each medium type in Attachment I.

5.2.5 Evaluate the samples at the end of the incubation period and record whether or not growth is recovered on the test plates, on Form 22712-01.

5.3 Growth Promotion

5.3.1 Obtain the media to be tested and allow them to equilibrate to room temperature.

5.3.2 Disinfect media containers and spreader packs prior to placing them in the BSC.

5.3.3 Select the appropriate challenge organisms according to Attachment 1: Media Types, Challenge Organisms, and Incubation Requirements.

5.3.4 Record the following information on Form 22712-01, Media Qualification Testing.

5.3.4.1 Medium type

5.3.4.2 BDP Lot number

5.3.4.3 Expiration date

5.3.4.4 Equipment information

5.3.5 Rehydrate the Quanti-Cult® microorganisms according to the manufacturer instructions below. If not using Quanti-Cult®, refer to the manufacturer's instructions.

5.3.5.1 Remove the number of rehydrating fluid vials and pouches of challenge organisms needed for testing from storage.

5.3.5.2 Record the following challenge organism information on Form 22712-01.

5.3.5.2.1 BDP Lot number

5.3.5.2.2 Lot number

5.3.5.2.3 Expiration date

5.3.5.3 Allow the challenge organisms to equilibrate to room temperature.

5.3.5.4 Warm the rehydrating fluid (blue cap) to 35-37°C.

5.3.5.5 Remove the vial containing the challenge organism film from the pouch.

NOTE: The cap contains an intact dried film of live organisms.

5.3.5.6 Remove and discard the blue cap when the rehydrating fluid is warm.

5.3.5.7 Remove the vial cap containing the challenge organisms and transfer it to the rehydrating fluid vial.

5.3.5.8 Tighten the cap.

5.3.5.9 Insert the vial into the foam rack.

5.3.5.10 Invert the rack and tap the vials to make sure the liquid is in contact with the inside of the cap.

NOTE: Each rehydrated Quanti-Cult® vial contains <100 CFU of the appropriate challenge organism in a 0.3 mL culture suspension.

NOTE: Each rehydrated Quanti-Cult Plus vial contains <100 CFU of the appropriate challenge organism per 0.1 mL of culture suspension. The Plus vials contain 1mL of culture, capable of running 10 test inoculations.

5.3.5.11 Incubate the vials in an inverted position, at 35-37°C for 15 minutes to suspend the preserved microorganisms.

5.3.5.12 Disinfect the vials after incubation and place them in the BSC.

5.3.6 Inoculation and Incubation of Solid Media Samples.

5.3.6.1 Label an un-inoculated plate as a negative control.

5.3.6.2 Label a media plate for each challenge organism as positive controls.

5.3.6.3 Label the test plates.

5.3.6.4 Inoculate the test plate with <100 CFU of the challenge organism by dispensing the appropriate volume or 100 µL in the case of BDP produced cell banks. Place the plate on a rotating stand and spread the organism while using a spreader in one hand and rotating the stand with the other hand.

5.3.6.4.1 In the instance that *Pichia pastoris* is to be used, a serial dilution of 10^{-6} (100µL inoculum volume) should be performed prior to plating. Sterile PBS, BDP PN 30007, should be used as the diluent for any dilutions.

5.3.6.5 Allow the medium to absorb the cell suspension.

5.3.6.6 Invert all the test samples including the negative and positive controls and incubate them according to the temperature and duration indicated in Attachment I. All control samples should be incubated at the same temperature and for the same duration as the media being tested. For qualifying bioburden cassettes, obtain lids for incubation of the cassettes from the accessory growth promotion funnel, BDP PN 31364.

5.3.6.7 If dual incubation temperatures are required, incubate all the samples at the lower temperature for the stated incubation period and then at the higher temperature.

5.3.6.8 Record the incubator identification numbers and calibration due dates on Form 22712-01.

5.3.6.9 Record the date and time incubated on Form 22712-01.

5.3.6.10 Count the number of colonies on each test plate following incubation and record the results on Form 22712-01.

5.3.7 Inoculation and Incubation of Liquid Media Samples.

5.3.7.1 Label an un-inoculated medium tube as a negative control.

5.3.7.2 Label a TSA plate for each challenge organism as positive controls.

5.3.7.3 Label the test samples.

5.3.7.4 Inoculate each test sample with <100 CFU of the challenge organism.

5.3.7.5 Incubate the test samples including the negative and positive control, at the temperature and duration indicated in Attachment I. All control samples should be incubated at the same temperature and for the same duration as the media being tested.

5.3.7.6 If dual incubation temperatures are required, incubate all the samples at the lower temperature for the stated incubation period and then at the higher temperature.

5.3.7.7 Record the incubator identification numbers and calibration due dates on Form 22712-01.

5.3.7.8 Record the date and time incubated on Form 22712-01.

5.3.7.9 Examine the samples for turbidity following incubation and record the results on Form 22712-01.

6.0 Acceptance Criteria

6.1 All negative controls and sterility samples do not demonstrate growth.

6.2 All positive controls plates recover growth of <100 CFUs .

6.3 Growth promotion test plates recover growth of <100 CFUs.

6.4 Liquid medium samples will be defined as “Shows Growth” when one or more of the following criteria has been met: media is turbid, a cell pellet is present, a media color change occurs, or any other growth defining change that can be observed by the unaided eye.

6.5 Sterile LB agar with 25 mcg/mL Kanamycin tested with DH5α does not recover growth. Growth is observed with DH5α pING-mTYRP2.

6.6 Sterile LB agar with 15 mcg/mL Tetracycline tested with DH5α does not recover growth. Growth is observed with BL21-AI.

6.7 Sterile LB agar with 20 mcg/mL Chloramphenicol tested with DH5α does not recover growth. Growth is observed with BL21(λDE3) pLysS.

6.8 Sterile LB agar with 25 mcg/mL Chloramphenicol tested with DH5α does not recover growth. Growth is observed with BL21(λDE3) pLysS.

6.9 Sterile LB agar with 50 mcg/mL Streptomycin tested with DH5α does not recover growth. Growth is observed with DH10B

7.0 Results Interpretation

7.1 Record the results as ‘Pass” if the test meets the acceptance criteria as stated in section 6.0.

7.2 Record the results as ‘Fail’ if the test does not meet the acceptance criteria as stated in section 6.0

7.3 Record the serial dilution used to achieve the observed result alongside the recorded CFU on Form 22712-01.

8.0 Definitions

8.1 **Colony Forming Unit (CFU) –** An individual cell which is able to clone itself into an entire colony of identical cells.

8.2 **Challenge Organism–** An organism that is specified to qualify media for growth promotion.

9.0 References and Related Documents

SOP 22153 *Bacterial Cell Bank Viability Assay*

Form 22712-01 Media Qualification Testing

21 CFR 610.12 (e) (2).

Current United States Pharmacopeia <71>.

10.0 Attachments

10.1 **Attachment 1** Media Types, Challenge Organisms, and Incubation Requirements

10.2 **Attachment 2** Instructions for Using Quanti-Cult®

11.0 Change Summary

Attachment 1
Media Types, Challenge Organisms, and Incubation Requirements

Type of Media	Challenge Organisms	ATCC Numbers	Incubation Temp and Duration
TSA agar for Environmental Monitoring	*A. brasil* *B. subtilis* *C. albicans* *E. coli* *P. aeruginosa* *S. aureus*	16404 6633 10231 8739 9027 6538	20-25°C 120-168 hrs 30-35°C 60-76 hrs
SAB agar for Environmental Monitoring	*A. brasil* *C. albicans*	16404 10231	20-25°C 120-168 hrs 30-35°C 60-76 hrs
TSA agar for Bioburden Testing	*A. brasil* *B. subtilis* *C. albicans* *E. coli* *P. aeruginosa* *S. aureus*	16404 6633 10231 8739 9027 6538	30-35°C 48-72 hrs
SAB for Bioburden Testing	*A. brasil* *C. albicans*	16404 10231	20-25°C 120-168 hrs
Plate Count agar for Bioburden Testing	*B. subtilis* *E. coli* *P. aeruginosa* *S. aureus*	6633 8739 9027 6538	30-35°C 48-72 hrs
YPD Agar for Colony Growth Testing	*P. pastoris* *C. albicans*	NA 10231	30-35°C 48-72 hrs
m-Endo LES agar for Coliform Testing	*E. coli* *P. aeruginosa*	8739 9027	30-35°C 24-72 hrs
TSB for Medium Fills	*A. brasil* *B. subtilis* *C. albicans* *E. coli* *P. aeruginosa* *S. aureus*	16404 6633 10231 8739 9027 6538	30-35°C 72-120 hrs

Attachment 1 (Continued)

Media Types, Challenge Organisms, and Incubation Requirements

Type of Media	Challenge Organisms	ATCC Numbers	Incubation Temp and Duration
LB agar without antibiotics	*A. brasil* *B. subtilis* *C. albicans* *E. coli* *P. aeruginosa* *S. aureus* DH5α	16404 6633 10231 8739 9027 6538 N/A	35-39°C 18-32 hrs
LB agar with 25 mcg/mL of Kanamycin	DH5α DH5α pING-mTYRP2	N/A	35-39°C 18-32 hrs
LB agar with 20 mcg/mL of Chloramphenicol	DH5α BL21(λDE3)pLysS	N/A	35-39°C 18-32 hrs
LB agar with 25 mcg/mL of Chloramphenicol	DH5α BL21(λDE3)pLysS	N/A	35-39°C 18-32 hrs
LB agar with 15mcg/mL of Tetracycline	DH5α BL21-AI	N/A	35-39°C 18-32 hrs
LB agar with 50mcg/mL of Streptomycin	DH5α DH10B	N/A	35-39°C 18-32 hrs