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## 1.0 Purpose

This procedure describes the assay methodology for determining the viability (cfu/mL) of bacterial cell bank samples. This procedure may also be used in conjunction with and prior to ***SOP 22155 - Antibiotic Resistance Testing and Typing Assays for Bacterial Cell Banks***.

## 2.0 Scope

This SOP applies to all release and R&D/FIO QC test requests for bacterial cell bank viability testing. Refer to the cell bank Master Specification and/or the Project Scientist for specific antibiotic medias and dilution ranges. Some cell banks may require antibiotic media and dilution ranges other than those indicated in this SOP. The use of alternative antibiotics or dilution schemes as directed by the Project Scientist or the Master Specification document must be noted on **Attachment 1** but will not require a PA test method deviation.

## 3.0 Authority and Responsibility

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

3.2 PA/QC personnel are responsible for the training and diligent performance of the procedures established in this SOP.

3.3 PA/QC personnel are responsible for completing required documentation per this SOP.

3.4 PA/QC Managers or designees are responsible for reviewing the data and documentation generated from this SOP.

3.5 Biopharmaceutical Quality Assurance (BQA) is responsible for quality oversight of this procedure.

## 4.0 Reagents and Materials

4.1 LB agar Plate (100 mm) with cover, without antibiotics, BDP PN 10446 or BDP approved equivalent.

4.2 LB agar Plate (100 mm) with cover + Kanamycin (25 (g/mL), BDP PN 10447 or BDP approved equivalent.

4.3 LB agar Plate (100 mm) with cover + Chloramphenicol (25 (g/mL), BDP PN 10449 or BDP approved equivalent.

4.4 LB agar Plate (100mm) with cover + Chloramphenicol (20 (g/mL), BDP PN 10450 or BDP approved equivalent.

4.5 LB agar Plate (100 mm) with cover + Tetracycline (15 (g/mL), BDP PN 10451 or BDP approved equivalent.

4.6 LB agar Plate (100 mm) with cover + Streptomycin (50 (g/mL), BDP PN 10448 or BDP approved equivalent.

4.7 Other defined media or antibiotic plates may be requested for specific projects, refer to project-specific documentation and PA Manager.

4.8 Disposable culture spreaders, sterile, BDP PN 20670 or BDP approved equivalent.

4.9 mL conical tubes, sterile, BDP PN 20006 or BDP approved equivalent.

4.10 mL Falcon tubes, sterile, BDP PN 20147 or BDP approved equivalent.

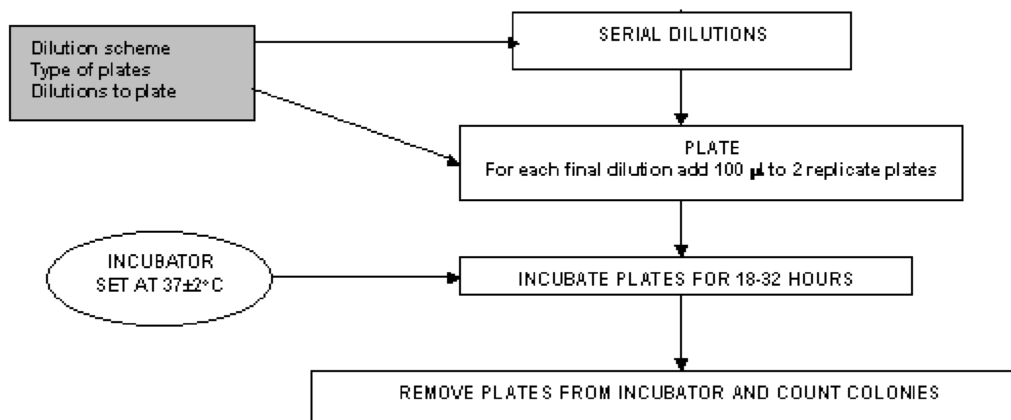
4.11 PBS solution, pH 7.4, BDP PN 30629 or BDP approved equivalent.

4.12 Parafilm, BDP PN 20464 or BDP approved equivalent.

4.13 Various Calibrated pipettes and pipette tips.

## 5.0 Procedure

5.1 Scheme for Bacterial Viability Assay.



5.2 Five 1-log serial dilutions of a test bacterial sample are used for plating duplicate media Petri dish plates. This range of serial dilutions provides for a sample viability range of 2 x $10^6$ to 4 x $10^{11}$ cfu/mL.

5.3 "Without antibiotics" are the minimum required set of LB agar plates for viability testing. For each type of antibiotic agar plate requested on QCTR Form 22002-01 or required by the Master Specification, use the same serial dilutions generated for the "without antibiotics" set for the "with antibiotic" plates.

5.4 **Negative Plate controls** - set aside one of each LB agar plate type required in the assay. Incubate the negative plate controls along with the viability plates.

5.5 **Negative Dilution Media control** - Spread 100 µL of PBS (whichever is used in the assay) on a single LB agar plate without antibiotics. Incubate the negative media control plate along with the viability plates.

5.6 **Negative E. coli Control Strain plate** – For each antibiotic and no-antibiotic media type used, perform a serial dilution of the *E. coli* DH5α negative control strain to $10^{-4}$ (as shown in Step 5.8). Spread one plate of each media with 100 µL of the $10^{-4}$ DH5α dilution to verify the presence of antibiotics in the media.

5.7 **Positive E. coli Control Strain plate** – For antibiotic media only, perform a serial dilution of the appropriate *E. coli* antibiotic resistant positive control strain to $10^{-4}$ (as shown in Step 5.8). Spread one plate of each media with 100 µL of the $10^{-4}$ positive control dilution to verify the presence of the desired antibiotic.

5.8 Serially dilute each sample into Falcon 2063, 6-mL dilution tubes or 15 mL conical tubes, with 900 µL PBS, pH 7.4 using the following chart: Gently vortex previous dilutions thoroughly before each subsequent dilution and immediately prior to plating.

| Dilution sequence | Steps | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| $10^{-1}$ $10^{-2}$ $10^{-3}$ $10^{-4}$ $10^{-5}$ $10^{-6}$ $10^{-7}$ $10^{-8}$ | **For $10^{-1}$** Add 100µL cells to 900µL PBS | **For $10^{-2}$** Add 100µL $10^{-1}$ diln. to 900µL PBS | **For $10^{-3}$** Add 100µL $10^{-2}$ diln. to 900µL PBS | **For $10^{-4}$** Add 100µL $10^{-3}$ diln. to 900µL PBS | **For $10^{-5}$** Add 100µL $10^{-4}$ diln. to 900µL PBS | **For $10^{-6}$** Add 100µL $10^{-5}$ diln. to 900µL PBS | **For $10^{-7}$** Add 100µL $10^{-6}$ diln. to 900µL PBS | **For $10^{-8}$** Add 100µL $10^{-7}$ diln. to 900µL PBS |

5.9 Starting with the $10^{-4}$ dilutions, spot 100 µL of the $10^{-4}$ to the $10^{-8}$ dilutions on pre-warmed (37 ± 2°C) agar plates in duplicate. Samples that contain ≤ 2 x $10^6$ cfu/mL will require lower dilutions to be plated in order to obtain valid results.

5.10 Using a Petri dish turntable, spread each dilution gently using a clean bacterial culture spreader.For disposable plastic spreaders, discard the spreader after each use (1 plate).

5.11 Spot and spread plates in sets no greater than four (4) plates at a time.

5.12 After spreading, cover and leave plates at room temperature for 30-60 minutes until PBS has been absorbed. Record the drying time on Form 22153-01.

5.13 Seal plates with Parafilm, invert and incubate plates at 37 ± 2 °C for 18 - 32 hours. Refer to the Project Scientist for the recommended incubation time. Record the incubation date and time, and the incubator temperature on Form 22153-01.

5.14 Upon completion of incubation, remove the plates from the incubator and record the date and time of removal as well as the incubator temperature on Form 22153-01.

5.15 Count all observed colonies on each plate and record on Form 22153-01. Plates that exhibit a bacterial lawn, are substantially confluent, or have > 400 colonies/plate, should be recorded as TNTC (Too Numerous to Count). Plates that exhibit no bacterial colonies should be recoded as "0."

**NOTE:** The negative control plates (PBS without sample) should not have any colonies following incubation. **If colonies appear on the negative control plates, the assay is considered invalid due to negative control failure. Investigate possible sources of bacterial contamination prior to repeating the assay.**

5.16 Average and record on Form 22153-01 the colony count for each set of replicate plates. For plates that exhibit a difference in colony counts greater than 50% of the averaged value of replicates, the dilution point is considered invalid. Note any invalid replicate sets on Form 22153-01.

5.17 Replicate plate averages < 20 or > 400 cfu/plate cannot be used to generate release results. Choose a dilution set that provides an average colony count between twenty (20) and 400 cfu/plate for calculation of sample cfu/mL.

## 6.0 Calculations

6.1 For the selected dilution with an average result between 20 and 400 cfu/plate, compute the average colony-forming units (cfu) per mL and record on Form 22153-01

6.2 cfu/mL = $\frac{\text{(avg. no. colonies per plate)}}{\text{(dilution plated)} \times \text{(mL plated)}}$

6.3 *Example*: For a sample with the following data:

Colony counts of 130 and 141, Average count = 135.5

Dilution plated: $10^{-2}$ (0.01)

Volume plated: 0.1 mL (100 μL)

The computation would be:

$$\frac{135.5}{10^{-2} \times 0.1} = 1.35 \times 10^{5} \text{ cfu/mL}$$

6.4 If more than 1 dilution provides an average between 20 and 400 cfu/mL, the dilution with an average value closer to 300 cfu/plate shall be considered the reportable result.

6.5 Record and calculate the cfu/mL values for each LB agar plate type used in the assay. The viability determined for the “without antibiotics” plates is calculated separately from that of each result for agar plate sets containing an antibiotic.

6.6 Complete and sign Form 22153-01 and submit for PA Manager review.

## 7.0 Acronyms

7.1 **Cam** – Abbreviation for the antibiotic Chloramphenicol.

7.2 **cfu** – Colony Forming Unit.

7.3 **Diln** – Abbreviation for dilution.

7.4 **Kan** - Abbreviation for the antibiotic Kanamycin.

7.5 **LB** – Luria-Bertani broth media.

7.6 **PBS** – Phosphate Buffered Saline.

7.7 **Str** – Abbreviation for the antibiotic Streptomycin.

7.8 **Tet** – Abbreviation for the antibiotic Tetracycline.

7.9 **TNTC** – Too Numerous To Count.

## 8.0 References and Related Documents

8.1 **SOP 22155** Antibiotic Resistance Testing and Typing Assays for Bacterial Cell Banks

8.2 **Form 22153-01** Bacterial Cell Bank Assay Calculations and Results

## 9.0 Change Summary