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1.0 Purpose

This method describes the procedure for determining the uniformity in appearance of a microbial culture's morphology. This method determines the appearance but does not differentiate individual colonies. These differences can be further examined using Gram Stain and Colony Identification. This method can be used to examine samples during or after a fermentation.

2.0 Scope

This Standard Operating Procedure (SOP) applies to all Process Analytics/Quality Control (PA/QC) personnel who will be determining the uniformity in appearance of a microbial culture's morphology.

3.0 Authority and Responsibility

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

3.2 Technical Operations Lead, PA/QC is responsible for training personnel in this procedure and for documenting this training to Biopharmaceutical Quality Assurance (BQA).

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

3.4 BQA is responsible quality oversight of this procedure.

4.0 Materials

4.1 Tryptic Soy Agar (TSA) plates, BDP PN 10006

4.2 Plastic, disposable inoculation loops (or equivalent), BDP PN 20675

4.3 Parafilm™ (BDP PN 20464) or Ziploc™ bags, BDP PN 20340

4.4 Laminar Flow Biosafety Hood

4.5 30°-35°C Incubator

4.6 2°-8°C Refrigerator

5.0 Procedure

Perform all the manipulations in a biosafety cabinet (BSC). Observe all principles of aseptic technique throughout the procedure. Samples are identified by their QC ID number and their description.

5.1 Warm the TSA plates to room temperature before use.

5.2 Warm the samples to room temperature before performing analysis.

5.3 Label individual plate bottoms with the date of the assay and the identifying numbers associated with each sample.

5.4 Each sample to be analyzed is streaked onto duplicate TSA plates so that after the incubation period, individual colonies can be observed. Uninoculated duplicate plates serve as a negative control. Any growth on these plates will invalidate the assay.

5.5 Allow the sample plates and negative controls to dry before they are placed into the incubator.

5.6 Parafilm the edges of each plate or put the plates into a Ziploc™ bag. Invert the plates and incubate the plates for seven (7) days at 30°-35ºC.

5.7 Daily (not including weekends or holidays) and at the end of the incubation period, remove all plates from the incubator and examine for growth. Document any differences in the appearance of the colonies on the plate. Note any growth on the negative control plates.

5.8 In addition to visual observation, the different colony morphologies may be submitted for speciation.

6.0 Documentation

6.1 Record observations taken daily from each of the duplicate plates on the Culture Morphology Form 22708-01. Note any differences in the appearance of the colonies on the plates.

6.2 Examples of Observations to Record on Form 22708-01.

- The colonies appeared to be uniform in morphology. There was no growth on the control plates.
- There appeared to be at least three distinct colonies growing on the plate. One appeared to be a large shiny brown colony, one appeared to be smaller and white in comparison, and the third appeared to be small and yellow. There was no growth on the control plate.

6.3 Review the PA/QC form before it is submitted to BQA.

6.4 Save the plates at 2°-8ºC for no more than 14 days in case speciation is requested for any isolate.

7.0 References and Related Documents

7.1 **Form 22708-01** *Culture Morphology*

[redacted]