

Department	Micro Laboratory	Document no	MICLAB – METHOD 011		
Title	Preparation of Bacillus spp Spore Suspension				
Prepared by:		Date:		Supersedes:	
Checked by:		Date:		Date Issued:	
Approved by:		Date:		Review Date:	

1.0 SUMMARY OF CHANGES

Version #	Revision History
MICLAB – METHOD 011	New

2.0 PURPOSE

This procedure details instructions for the preparation of Bacillus spp spore suspension.

3.0 SCOPE

This procedure is to be used when preparing a Bacillus spp spore suspension for microbiological testing in the laboratory (eg. product inoculation, TMV, antibiotic assays, inhibition tests, sterilization biological indicators).

4.0 RESPONSIBILITY \ BUSINESS RULES

This procedure applies to all Microbiology Laboratory staff.

5.0 PROCEDURE

5.1 Materials Required List

- 5.1.1 Chosen Bacillus spp. strain
- 5.1.2 Tryptone Soya Broth (TSB) in MacCartney bottles
- 5.1.3 Tryptone Soya Agar - TSA (large slope and bulk for pouring plates)
- 5.1.4 Pipettes – sterile
- 5.1.5 Petri-dishes sterile
- 5.1.6 Inoculating loop 10 μ L
- 5.1.7 Incubator 30°C \pm 1°C, 37°C \pm 1°C
- 5.1.8 Water bath
- 5.1.9 Centrifuge and centrifuge tubes (sterile)
- 5.1.10 Phosphate buffer - pH 7.2 (prepared by QC laboratory)

5.2 Procedure

- 5.2.1 Open freeze dried ampoule (of chosen Bacillus spp. strain).
- 5.2.2 Incubate at 37 \pm 1°C for 48 hours.
- 5.2.3 Streak onto TSA to check purity.
- 5.2.4 If pure, streak onto a large TSA slope and incubate at 37 \pm 1°C for at least 7 days.
- 5.2.5 Check spore production every 3 days by performing spore stain until approximately 80% of the cells yield spores.
- 5.2.6 Wash off the growth with 30mls Phosphate Buffer pH 7.2 and dispense into sterile centrifuge tubes.
- 5.2.7 Centrifuge the tubes at 1000 r.p.m. for 10 minutes. Decant the supernatant liquid.

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- 5.2.8 Wash pellet three times in fresh Phosphate Buffer and combine harvested spores.
- 5.2.9 Heat shock the suspension following procedure GM062932 Step 3.6 and 3.7.
- 5.2.10 Enumerate the number of viable spores. (Usually contain about 10⁹ spores per mL)
- 5.2.11 Pour plates with TSA and incubate at appropriate temperature depending on species (GM062932 Step 3.10) for 2 days.
- 5.2.12 Dispense the spore suspension into a sterile plastic container and keep refrigerated at 2-8°C.
- 5.2.13 Label container containing spore suspension with strain number, date of preparation, count/ml and date of expiry which is 6 months from date of preparation.

6.0 **DEFINITIONS / ACRONYMS**

N/A