Department	Micro Laboratory		Document no	MICLAB – METHOD 002	
Title	Enumeration of Biological Indicators				
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1. SCOPE AND APPLICATION

This general test method applies to the enumeration of viable spores on spore strips. This procedure will be used to evaluate suitability of spore strips for use in monitoring sterilisation efficacy of autoclaves.

2. REAGENTS AND MATERIALS REQUIRED

2.1 Glass beads

2.2 McCartney bottles

2.3 Sterile distilled water

2.4 Water bath

2.5 Vortex

2.6 Tryptone Soya Agar (TSA)

2.7 Stop watch

2.8 Petri dishes

2.9 Pipettes - sterile

2.10 Diluent 9.0 mls and 9.9mls (0.1% peptone water)

2.11 Thermometer

3. GENERAL TEST PROCEDURE

3.1 Place 5 or 6 glass beads (5mm in diameter) inside McCartney bottle.

3.2 Sterilise by autoclaving at 121°C for 30 minutes.

3.3 Transfer the spore strip to a sterile McCartney bottle containing glass beads.

3.4 Add 10 ml of sterile distilled water. (This is now a 1/10-dilution ie. 10^{-1})

3.5 Vortex on high until a homogenous pulp is obtained.

3.6 Heat shock the suspension in a water bath.

Bacillus pumilus	at 65°C to 70°C for 15 minutes.
Bacillus subtilis var. niger	at 65°C to 70°C for 15 minutes.
Bacillus stearothermophilus	at 95°C to 100°C for 15 minutes.

Place McCartney bottle containing 10 mls of distilled water alongside the test sample. Insert thermometer into the control McCartney bottle.

Start timing when the temperature inside the control bottle reaches the required temperature.

3.7 Cool rapidly in ice water.

3.8 Enumerate the suspension by preparing serial dilutions in 9.0 or 9.9 mLs of diluent.

3.9 Plate out -4, -5 and -6 dilution in duplicate by treating 1mL of the homogenised spore suspension as a 1/10 dilution of the spore strip (ie. 10^{-1}). Pour plates with approximately 15 ml Tryptone Soya Agar. Allow agar to solidify.

3.10 Place plates in a stomacher bag to avoid drying out of plates.

Incubate plates in inverted position.

Bacillus subtilis var. niger	at 37± 1°C for 2 days.
Bacillus pumilus	at 37± 1°C for 2 days.
Bacillus stearothermophilus	at 55± 1°C for 2 days.

Note: If plates are incubated at 55°C, place pates into a closed container to prevent agar drying out.

4. CALCULATION OF RESULTS

Count plates and record results on SF150138. As the homogeneous pulp was a 1/10 dilution of the spore strip the result after multiplying by the dilution factor is expressed per spore strip.

	Dilution		
e.g.	10^{-4}	10^{-5}	10^{-6}
	240	24	2

$\therefore$ 2.4×10^{6} cfu/ spore strip

Counts obtained should be within 0.3 logs of the stated population.

Confirm identity of isolates by checking spore positions and colony colour and identify up to genus level by setting up API 50CHB:

Bacillus subtilis var. niger – orange colonies, spores ellipsoidal, central with no swelling of the sporangium.

Bacillus pumilus – cream colonies, spores ellipsoidal, central with no swelling of the sporangium.
Bacillus stearothermophilus – cream colonies, spores ellipsoidal, central, which may or may not have swelling of the sporangium.

5. REFERENCE DOCUMENTS

5.1 The Microbiological Update for Pharmaceuticals, Medical Devices and Cosmetics Vol.18 No.11 February 2001 Editor, Murray S Cooper, Phd.

5.2 USP 24-NF 19 Supplement 2

6. REVIEW HISTORY

Version #	Revision History
MICLAB – METHOD 002	New

