Department	Micro Laboratory		Document no	MICLAB – METHOD 032	
Title	YEAST AND MOULD COUNT IN MICROBILOGICAL LABORATORY				
Prepared by:		Date:		Supersedes:	
Checked by:		Date:		Date Issued:	
Approved by:		Date:		Review Date:	

1. SCOPE AND APPLICATION

This general test method shall be followed in order to determine the total yeast and mould count. This procedure applies to manufactured products, raw materials used in manufacture and environmental samples at a Microbiology Laboratory of GMP site.

2. REAGENTS AND MATERIALS REQUIRED

2.1 Sabouraud Dextrose Agar (SDA) - prepared plates or molten agar

2.2 Tryptone Soya Broth plus 4% Tween 80 (TSB+T) or other neutralising broth as predetermined by validation study.

2.3 Stomacher bags.

2.4 Pipettes - sterile

2.5 Sterile plastic disposable spreaders

2.6 Petri dishes

3. GENERAL TEST METHOD

3.1 Weigh out 10g of sample into the stomacher bag.

3.2 Make up to 100g with TSB + T (or other neutralising broth)

3.3 Homogenise well by placing it in the stomacher for 15-30 seconds. If weighed directly into a bottle shake well (This is a 1 in 10 dilution).

3.4 If the recovery sensitivity according to the validation data is less the 10, then plate out 1mL of the 1 in 10 dilution in duplicate and pour with molten SDA. Allow to set.

3.5 If the recovery sensitivity according to the validation data is less than 100 spread plate method, then plate 0.1mL of the 1 in 100 dilution in duplicate onto prepared and dried SDA plates.

3.6 Spread evenly on the agar surface using a disposable sterile plastic spreader.

3.7 If the recovery sensitivity according to the validation data is less than 100 pour plate method, then plate out 0.1mL of the 1 in 10 dilution in duplicate and pour with molten SDA. Allow to set.

3.8 Incubate plates in the upright position for 5 days at 25 ± 1°C. Plates should be stacked no more than six high.

3.9 At the end of the incubation period remove plates from the incubator and count the colonies of yeast and mould separately. Count colonies on plates that yield between 15 and 150 forming units (CFU) per plate.

NOTE:
If the number of colonies per plate is more than 150, the result should be reported as greater than 150 times the dilution factor. For counts less than 15, the actual number of colonies on the lowest dilution should be reported as the estimated colony count of colony forming units (cfu's) per gram

3.10 Multiply the count by the inverse of the dilution factor and report as total yeast and mould count per gram of sample tested.

3.11 Report all results to two significant figures.

Eg: 1340 = 1.3×10^3
1350 = 1.4×10^3
1370 = 1.4×10^3

3.12 If high fungi counts are expected, in addition to the above dilution, prepare serial dilutions by adding 1ml of initial dilution into 9ml of sterile 0.1% peptone diluent. Plate out dilutions as required using the technique described above.

