

Standard Operating Procedure

Title: Microbiological Evaluation of Bioburdens, Non-Sterile Products and Raw Materials

The following method is used for IPA samples and all growth is to be evaluated:

- 7.1 Samples must be collected in sterile 100mL bottles. Filter the sample (100mL) through a sterile 0.45µ Hydrophobic Membrane filter. Wash the filter with 150mL of sterile Peptone Water. Aseptically transfer the filter into a 100mL bottle containing 100mL of sterile Letheen Broth.
- 7.2 Incubate bottle at 32°C for 5 days. If growth is observed, subculture onto a Nutrient agar plate and incubate for 48 hours at 32°C. All colonies are to be gram stained.
- 7.3 Record the results in appropriate log book.
Note: When entering IPA batches into record book, a batch number must be allocated.
Alert Level for prepared IPA: Zero organisms/100mL.

8 Speciation Procedures for Organisms found in Non-Sterile Products and Raw Materials

- 8.1 If after incubation micro-organisms are apparent in either the bottle/s of medium or Agar plates for any Non-Sterile Product or Raw Material tested for the Presence of Micro-organisms and/or Microbial Limit Test, the following steps should be carried out:
 - a) The fact that growth has occurred and in which medium and the time period involved, and the number of colonies present, should be recorded into the appropriate Recording System.
If further ID is required, according to the control or raw material method specifications:
 - b) The organism/s present is/are to be streaked out (for isolated colonies) onto a Nutrient Agar plate and incubated at 32°C for 48 hours. If growth has occurred in the FTM also streak the contaminant onto Reinforced Clostridial Agar plate and incubate at 32°C in an anaerobic jar for 48 hours.
 - c) A Gram stain of the different organisms present must be carried out and details of their microscopic and macroscopic appearance recorded appropriate Recording System.
 - d) A retest on the product may have to be carried out - details are given in Section 10, "Retest Procedures"
 - e) If the following organisms are found, take the action detailed below:
 - 8.1.1 Gram Positive rods or Moulds
Take no action unless they are in excess of the Action Level specified for each product or Raw Material or they are shown on retest to have increased in number.
 - 8.1.2 Gram Negative cocci
Inform the Microbiology Manager if GNC found in product. Further investigation and Identification is required, including a KOH test to confirm that the organism is Gram negative.
 - 8.1.3 Yeast
The requirement is for absence of Candida albicans. If the yeast is shown to be other than Candida albicans take no action unless they are in excess of the Action Level specified for each product or Raw Material or they are shown on retest to have increased in number.
Note: See MICLAB 070 for Identification of Candida albicans.
 - 8.1.4 Gram Positive Cocci
The requirement is for absence of Staphylococcus aureus. Heavily streak the suspected organism onto a Mannitol Salt Agar plate and incubate at 32°C for growth for a maximum of 3 days. At the same time streak the suspected organism onto a Nutrient Agar plate and incubate at 32°C for 48 hours. (In the case of needing to perform the test.) Presumptive coagulase - positive Staphylococci produce colonies with bright Yellow zones, while (non-pathogenic) coagulase-negative Staphylococci are surrounded by a Red or Purple zone.