

Standard Operating Procedure

Title: Aseptic Media Filling and Micro. Integrity Leak (Soup) Testing Procedure

- 4.2. The filled units received by the Microbiology Laboratory are to be placed into the 30°C (±1.5) Hot room. The Microbiology Laboratory personnel are to inspect the containers for evidence of microbial growth after 7 and 15 days incubation. During the 7day inspection, shake the

NORMAL VALUE	Zero units contaminated
ALERT LIMIT	One contaminated unit, raise a DR and start an investigation and consider a repeat media run.
SHUTDOWN LIMIT	Two contaminated units, raise a DR and start an investigation and commence revalidation of the process with 3 consecutive successful media runs

contents of the container to ensure that the media has come in contact with all internal surfaces of the container. Store back into either shippers or buckets in a different orientation to the first incubation period. Record all additional information onto the "Aseptic Media Fill Information Sheet" (**Form670**) and in the media fill Manufacturing Instruction sheets, which were initiated at the manufacture of the medium used for the media run.

- 4.3. If any containers show evidence of microbial growth raise a DR. Inform Microbiology Manager, Production Manager and review the possibility of off line dye testing to confirm container integral. Then open the container and streak (for individual colonies) the contaminated broth onto a Nutrient Agar plate and incubate at 30°C (±1.5°C) for 24 hours.
- 4.4. If the contaminant/s is a bacteria or yeast, identify to Genus level and if possible species level (**MICLAB 070**). Record the details of morphological appearance Gram stain reaction and identity (if determined) on Form 670 and in the Media Fill manufacturing instruction sheets.
- 4.5. After the required incubation time (15days) the medium must be checked for its ability to promote the growth of low levels of microorganisms. Under Laminar Flow, aseptically pool the medium from several containers into at least 6 sterile 100mL bottles or if possible, e.g. with vials, directly inoculate the media-filled containers with microorganisms as detailed below.

Inoculate 1/3 of these bottles with *Staphylococcus aureus* at levels of ideally 10-20, but definitely less than 100 viable organisms and incubate at 30°C (±1.5°C).

Inoculate a further 1/3 of the bottles with *Candida albicans* at a similar level and incubate at 30°C (±1.5°C). Inoculate the final 1/3 with *Bacillus subtilis* at a similar level of viable spores and incubate at 30°C (±1.5°C).

Note At least once per year, for a media run conducted on each piece of filling equipment, substitute an environmental organism isolated from the Sterile filling area for one of the above micro-organism.

Examine these bottles daily for evidence of microbial growth.

Growth should be evident in all the bottles within 48 hours incubation. The inoculum can be obtained by appropriate dilution of the stock suspensions of the organisms held in the Microbiology Laboratory refrigerator and the level used checked by plate count. See MICLAB 090.

Record results of the medium's ability to promote the growth of microorganisms on Form 670 and also in the Media Fill manufacturing instruction sheets.

4.6. Acceptance Criteria– the target is zero positives

The target should be zero growth but a contamination rate of less than 0.1% with 95% confidence level is acceptable