Standard Operating Procedure

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

Related Documents

Form 665	Microbiological Integrity (Soup) Test
Form 670	Aseptic Media Fill Information Sheet
MICLAB 020	Destruction of Biological Waste in the Microbiology Laboratory
MICLAB 040	Aseptic Media Filling and Soup Test Guideline
MICLAB 070	Identification of Microorganisms to Genus and Species Level
MICLAB 090	Stock Suspension of Micro Organism

EHS Statement

- All care must be taken when preparing media fills with HOT water. Rubber gloves are to be worn when using HOT water.
- Safety glasses and gloves are to be worn when using 70% IPA.

Table of Contents

1. Introduction

The procedures set out in this SOP should be carried out TWICE every year on all the filling equipments, at defined 6 monthly intervals on each production shift. Three successful media fills should be conducted as part of the validation/ commissioning of any new piece of equipment or after any significant re-design of existing equipment. (See MICLAB 040) To demonstrate container integrity in a new process or after any significant change that may affect the integrity, Microbiological Integrity Leak test ("Soup" test) may be required, (**Form 665**).

2. Procedure

2.1. A representative from the Microbiology Laboratory team is to attend the scheduled meeting the week prior to a media run being performed on a process. At this meeting they will outline the purpose of the media run, the type and size of product container to be used and hence the volume of medium required (determining a minimum number of units to be filled with sufficient medium), the volume required is dependent on the process to be evaluated. They will go over the Intervention matrix to predetermine what routine interventions and non-routine interventions need to be conducted during the media run.

Refer to Appendix 1 for Intervention matrix.

2.2. The Microbiology Laboratory staff will arrange for sufficient dehydrated Tryptone Soy Broth medium (as required).

2.3. The Microbiology Laboratory staffs are to initiate filling in **Form 670** for each batch of media which then moves with the process flow of the media run and is returned from production when completed and is to be stored in the "In-Process Media Run" file. The documentation is to be completed and if there is no data to be entered then N/A must be written in the space provided.

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

Microbiological Integrity (Soup) Test
(Ref. MICLAB 035)

Filling Machine and Number	
Container Type and Size	
Reason for "Soup" Test	
Number of Units to be Tested	
Initial Date of Test	

Acceptance Criteria:

Not more than 1 in 1000 units to show evidence of microbial contamination.

Equipment Required:

In "Soup Room":

44 Gallon drums on wheel trolleys
Circular mesh lids
Lead divers weights
Nylon netting bags
18mm hose with tap fittings
Drum Outlet valve hoses
Orange Crates

From Microbiology Laboratory:

3kg TSB powder per test drum (5 x 600gm bags / per drum)
1 bottle per test drum of 18hr *E.coli* culture in 250ml TSB
Rubber gloves
Rubber boots
Hibitane
Face-masks
Hair-nets
Thermometer

"Soup" Test Method: Day 1 Date Commenced: _____

- Turn on hot water system, fan and heater.
  Check that the "Soup Room" is at approximately 32°C.
- Prepare 1 x 250ml TSB bottle inoculated with an *E.coli* culture, per test drum.

"Soup" Test Method: Day 2 Date Commenced: _____
(for each test drum)

Aseptic Media Fill Information Sheet

(Ref. MICLAB 035)

Start Date _____

Reason for Media run:	

Micro to Complete

Machine Number	
Batch number	
Code	
Media type & Lot No. and Expiry Date	
Volume of media prepared & Storage Vessel size	
Time media prepared	
Media Prepared by	
Manufacturing instruction sheet filled up?	
Container & Size	
Volume fill size	
Soup Test required	YES / NO

Production to complete

Machine/Line No.	
Line steamed and date	
Machine Steaming and date	
Holding Tank # & Steaming	

Aseptic Media Fill Information Sheet

(Ref. MICLAB 035)

Sterilisation/Product Filter change			
Major Stoppage			
Electrically Adjustments			
Mechanical Adjustments			
Recovery Procedure Stop, Clean, Flush			
Mould head change			
Cleaning of mould and extruder			
Parison and bag adjustments			

Aseptic Media Fill Information Sheet

(Ref. MICLAB 035)

Stasis Media Check as per MICLAB 035

Organism	Date	Inoculum	Bottles	Temp.	Result
S.aureus *ATCC 6538*			x 2	30º	
C.albicans ATCC 10231			x 2	30º	
B.subtilis ATCC 6633			x 2	30º	
A.niger ATCC 16404			x 2	30º	
E.coli ATCC 8739			x 2	30º	
Environmental Isolate			x 2	30º	

Comments:

