Standard Operating Procedure

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

Related Documents

MICLAB 065	Determination of Heat Resistance of Spore Forming Organisms
MICLAB 060	Micro Laboratory Procedure for Sterility Testing
MICLAB 070	Identification of Micro organisms to Genus and Species Level
MICLAB 095	Sterile Sampling Procedure for Microbiology Laboratory

EHS Statement

- Correct Aseptic technique must be used when performing any Microbiological procedures.
- Safety glasses and gloves are to be worn when using IPA/Solvent.
- All testing must be preformed within a LAF cabinet.

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1 Equipment preperation For Non-sterile testing:

1.1 Assemble filtration units together.

1.2 Place 6 filtration units in a dacron bag, place autoclave tape with Date Autoclaved on the tied section.

1.3 Place 6 forceps inside individual test tubes, then place the 6 filled test tubes inside a Sterilope bags, seal by folding over twice and applying autoclave tape. Add the Number and Item and Date Autoclaved on the front of the bag.

1.4 Follow the above procedure for preparation of spoons and scissors.

1.5 Place the wrapped bags of forceps, scissors and spoons into the wire rack for the autoclave.

1.6 Autoclave the items in laboratory autoclave.

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If count obtained is over the Alert Level of 100 and less than 200 colonies, conduct a Gram stain on different types of colonies present.

If count obtained is over 200 colonies and not countable a retest is to be performed. Gram stain is required on the original sample, which is over the Alert Level.

If any Gram negative rods are present, identify for conformance. Record all results in log book.
If a retest is required, apply the same test method to the original sample using 10mL and 1mL volumes so that an exact count is obtained.

If a re-retest is required apply the same test method to the original sample using volume less than 1ml, i.e. 0.5ml and 0.1ml as to obtain a count.

After reading and recording the result in the log book, the BSB bottles can be cleaned out of the refrigerator,

OR either retested.

Every 6 months, graphical representations of Bioburden results are to be printed and stored in the Bioburden Reports File.

If the Bioburden is more than alert level, inform the Microbiology Manager who will decide if any additional testing of the product is required, e.g. LAL Pyrogen test. The reason for release is to be added in the 'Comments' section of the log book.

4 Filled Container Bioburdens (FCB)

4.1 Example-Prior to Autoclaving

All batches manufactured are to be sampled for Filled Container Bioburden testing.

4.1.1 Predetermined units are to be randomly selected immediately prior to the autoclaving of the final load. These samples are to be labelled "Bioburden" and placed into a plastic bag and delivered to the Micro. Lab. (**see MICLAB 095**).

4.1.2 Aseptically pool specified amount from each of the units into a sterile Membrane Filtration unit and filter through a 0.45µ membrane filter and wash with sterile Peptone Water. Transfer the filter into a sterile petri-dish containing an absorbent filter pad moistened with Tryptone Soy Broth.

4.1.3 Incubate at 30-35°C for 5 days under humid conditions. If any colonies are present Gram stain & record the morphological and microscopic appearance. If any Gram-positive or negative rods are present, identify further.

4.1.4 Record the results in the log book. Specify Alert and action limits.

5 Raw Material Bioburdens, (RMB)

Sterile operator's sample raw material for microbiological analysis and the samples are either delivered directly to the laboratory or placed into the micro sample cabinet within production.

6 Surgical Face Masks

Every batch of Face Masks purchased must be examined for microbiological status prior to use. On delivery of every batch, 5 boxes of facemasks will be randomly sampled by the Warehouse Sampling staff, and delivered to the Microbiology Laboratory. Test as per Raw Material Specification and test method.

7 Isopropyl Alcohol (70% IPA)

Isopropyl Alcohol is routinely used throughout the Microbiology Laboratory. The Production Services team prepares the 70% IPA. A sample from each new keg is to be aseptically sampled into a sterile 100ml bottle and sent to the Micro Lab for testing,

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

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- Correct collection, storage and preparation of test sample and collection vessel.
- Correct set-up of test equipment.
- Correct method used for test.
- Completed Training records.
- Expiry of consumables and equipment observed.
- Media stasis satisfactory.
- Correct incubation conditions of test.
- Observations made during test procedure.
- All calculations checked.
- All test control results, where relevant, are satisfactory.
- Review of other test results for the test session, to determine a trend.

The above items must be clearly addressed in the DR for OOS results

9.2.2 Phase 2 investigations involve a thorough systematic analysis of the incident, to determine the root cause for the OOS result. Phase 2 investigations are to be tailored for each incident to reflect the significance of meeting or exceeding either the ALERT or ACTION Levels.

9.3 Phase 2 Investigation Responses

9.3.1 Determine if the alert excursion is to become an action level excursion that requires upgrading the notification to an action level based on the following criteria:

9.3.2 Three alert/action level excursions from the same sterile area on the same day.

10.3.3 The minimum requirements for most situations are listed as below, this list is by no means exhaustive and specialist assistance from departments other than Microbiology should be utilised in the investigation.

10.3.4 All affected departments will perform their part of the investigation, carry out corrective actions and findings of the investigation.

9.4 OOS Bulk Solution Bioburden

9.4.1 Alert Level

Results obtained over the alert level require the following actions

- Gram stain
- Further identification as outlined in section 9 'Specification Procedures for Organisms found in Non-Sterile Products and Raw Materials'

9.4.2 Action Level (In addition to alert level requirements)

Results obtained over the action Level require the following details

- Results of any material used to manufacture the batch e.g. WFI & raw materials
- Sterilisation of vessels (SIP)
- Cleaning records for vessels (CIP)
- Review of other test results to determine a trend.
- Environmental conditions conform
- CAPA recommendations

9.5 OOS Filled Container Bioburden

9.5.1 Alert Level

Results obtained over the alert level require the following actions

- Gram stain
- Further identification as outlined in section 9 'Specification Procedures for Organisms found in Non-Sterile Products and Raw Materials'
- D-values where applicable.

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9.8.2 Action Level (In addition to alert level requirements)

Results obtained over the action Level require the following details

- Additional testing may be required.
- Put masks on hold and hold an investigation meeting.
- Contact supplier.

9.9 OOS IPA

9.9.1 Alert Level

- Gram stain
- Further identification as outlined in section 9 'Specification Procedures for Organisms found in Non-Sterile Products and Raw Materials'

9.9.2 Action Level (In addition to alert level requirements)

Results obtained over the action Level require the following details

- Additional testing may be required.
- Contact immediately to ensure IPA is not distributed to production.
- Contact immediately to resample the batch.

10 Retest and Repeat Procedures for Non-Sterile Products and Raw Materials

10.1 Repeat Procedures

A test may be repeated when the initial test has been invalidated due to an error in the procedure (including equipment, Technician, etc).

10.2 Retest Procedures

Where the number of micro-organisms in the Non-sterile product or Raw Material detected in the Microbial Limit Test is over the ALERT LEVEL for that material, a retest on the Non-Sterile product or Raw Material in question should be carried out. The retest involves repeating the Microbial Limit Test in triplicate, for both Limit and Presence testing. If after retesting the product or raw material three times the number of micro-organisms is still over the ALERT LEVEL the situation is to be evaluated by the Microbiology Manager.

Note:
If there is not a sufficient quantity remaining from the original sample for either repeat test or the re-test, new samples should be acquired by randomly sampling throughout the batch.

11 Summary of Changes

Version #	Revision History
MICLAB 075	New

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