

Standard Operating Procedure

Title: Environmental and Plant Hygiene Monitoring Procedure

| | |
|-------------------|--|
| MICLAB 070 | Identification of Microorganisms to Genus and Species Level |
| MICLAB 075 | Micro Evaluation on Bioburden, Non sterile and Raw Materials |
| MICLAB 095 | Sterile Sampling Procedure for Microbiology Laboratory |
| MICLAB 100 | Microbiological Testing of Compressed Gasses |

EHS Statement:

- Care must be taken at all times while collecting samples.
- **Safety glasses and gloves** must be worn at all times when using 70% IPA or solvent.

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Procedure

Daily monitoring of sterile grade areas during production is to be conducted by trained production staff. The Microlab is to ensure that the necessary plates are delivered on a daily basis so monitoring can take place.

Once a test has been completed, the responsible operator is to initial the plate and make sure that the BPN of the batch running at the time of the test is written on the plate. Plates will be labelled with prompts to ensure this isn't forgotten. If no batch is running at the time of the test N/A should be put on the plate instead of a BPN.

If an area of concern is noted during routine daily testing, inform Micro immediately so that further steps can be taken.

Once a week a Microlab technician will perform environmental monitoring and a housekeeping audit of the area.

If, in monitoring any of the following areas, any of the following events occur, the Microlab staff member responsible for conducting Environmental Monitoring in that area is to launch an Environmental Monitoring Investigation by IMMEDIATELY repeating the test and as follows:

Table A

| | EVENT | ACTION |
|----|---|--|
| 1. | The <u>action level</u> is exceeded (defined as twice the Alert Level). | DR to be raised and an OOL Investigation started. Notify the Microbiology Manager /Area Manager and/or Service Engineers/Cleaners. |
| 2. | Three consecutive monitoring results are above the Alert Level. | DR to be raised and an OOL Investigation started. Notify the Microbiology Manager /Area Manager and/or Service Engineers/Cleaners. |
| 3. | The result of a <u>trend analysis</u> over a period | DR to be raised and an OOL Investigation started. |

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| Sterile filling | 1000L | 32°C (± 1.5 °C) 48 hours (+ 24 hrs) | Total count Re-incubate DR and Repeat testing if required. | 25°C (± 1.5°C). 72 hrs (+ 48 hrs) | Total count, and indicate # of mould. Gram Stain/ID DR and Repeat testing if required. |
| Sterile preparation | 200L | 32°C (± 1.5 °C) 48 hrs (+ 24 hrs) | Total count Re-incubate DR and Repeat testing if required | 25°C (± 1.5°C). 72 hrs (+ 48 hrs) | Total count, and indicate # of mould. Gram Stain/ID DR and Repeat testing if required. |
| Non Sterile | 200L | 32°C (± 1.5 °C) 48 hrs (+ 24 hrs) | Total count Re-incubate DR and Repeat testing if required | 25°C (± 1.5°C). 72 hrs (+ 48 hrs) | Total count, and indicate # of mould. Gram Stain/ID DR and Repeat testing if required. |
| Non Sterile | 200L | 32°C (± 1.5 °C) 48 hrs (+ 24 hrs) | Total count Re-incubate DR and Repeat testing if required | 25°C (± 1.5°C). 72 hrs (+ 48 hrs) | Total count, and indicate # of mould. Gram Stain/ID DR and Repeat testing if required. |

Air sampling must also be conducted in all of the Filling rooms in the Sterile Area prior to, or as close as possible to the commencement of filling processes after any non-aseptic procedure has been carried out in a Sterile Filling area, i.e. After "open day" maintenance, etc. Any deviation from this SOP is to be recorded in the "Environmental Monitoring Comments" book and a DR raised, if necessary.

2. Micro-Organism levels on surfaces using Contact Plates

- 2.1. Prepared irradiated and non-irradiated Count-Tact plates purchased from an approved supplier are to be used in the monitoring of surfaces inside the factory. Use irradiated plates in sterile grade areas and non-irradiated plates in non sterile grades. If plates are not available prepare contact plates with approximately 18mL/plate of **Nutrient Agar + 3% w/v Tween 80**. Ensure plates are sufficiently full to allow a convex agar surface above the level of the plastic base.
- 2.2. Prepared Count-Tact plates will have an expiry date printed on the surface of the lid. All other plates prepared with in the microlab have an expiry date of 1 month from date of pouring. Store plates on compactor shelves.
- 2.3. Procedure for sampling - Operator to assess for "Worst Case" situation:
 - 2.3.1. Record details of date and area sampled on the contact plate.
 - 2.3.2. Touch agar surface of plate to surface to be sampled and hold plate down for 10 seconds.
 - 2.3.3. Replace the lid on the plate.
 - 2.3.4. Spray area sampled with 70% Isopropyl alcohol (IPA) and wipe with a clean wipe.
 - 2.3.5. Sign and date relevant form after each area is sampled
 - 2.3.6. Record any unusual activities/observations about the area being monitored in the Environmental Monitoring Comments book, include date and time.
 - 2.3.7. Incubate plates inverted at 32°C (± 1.5°C) for 48 hours (+ 24 hours).
 - 2.3.8. After incubation the total count is recorded on a histogram in either the Environmental Results Surface Non- Sterile or the Sterile File. Prepare separate graphs for each area sampled. Alert and Action Limits need to be ruled onto the form in red pen and the location written on the top of the form next to Area. Once a

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- 3.1.3. When exiting the Sterile Scrub Preparation Room fill in **Form 635** the Daily Personnel Monitoring Log for Sterile Areas.
- 3.1.4. Collect the Lethen Agar plates from the factory after checking them against **Form 635** and incubate in the Microbiology Laboratory.
- 3.1.5. Incubate plates, inverted, at 32°C (± 1.5°C) for 48 hours (+24 hours). After incubation, examine the plates and count the number of colonies present.
- 3.1.6. Record the details of the number of colonies graphically in the Personnel Monitoring Reports files. Prepare separate graphs for each Operator. Record the total count as well as number of moulds present on the histogram graphs shading them in with a Green pen. For the purpose of determining if the count is over the specified limit, moulds are included in the final result.
- Record the details of the Microlab Technician results on the Monitoring results for sterility test sessions Finger, Fallout, Environmental and Personnel Results forms.
- 3.1.7. Limits

Sterile Manufacturing.

Microlab Technician each sterility session.

| Limits for Microbial contamination (average values) refer GMP guide lines | | |
|---|--|--|
| Grade | Glove Print <u>5 fingers</u> cfu/glove Alert Limit | Glove Print <u>5 fingers</u> cfu/glove Action Limit |
| Sterile Filling | <1 | <1 |
| Sterile Preparation | 3 | 5 |
| Packing | - | - |

On the Manufacturing graphs the Alert limit will be shown as 6 and Action limit will be 10 to account for both hands. In the event that the result from the Finger dab monitoring equals or exceeds either the ALERT or ACTION levels, follow the actions as listed under 'Micro Organisms on Personnel', see Section 9.

3.2. Operator's Clothing

The number of viable microorganisms on the clothing of Sterile Area Operators is to be enumerated daily for every batch using contact plates.

At the completion of Sterility Testing the Microlab technician is to perform testing of their clothing.

Note: If a double consecutive session is being tested, glove print impressions should be taken after the first session with gloves then changed before the second session. Personnel uniform monitoring should be performed after the second session only.

The method to be used is as follows:

- 3.2.1. Prepared irradiated Count-Tact plates are purchased from an approved supplier. If plates are not available prepare contact plates with approximately 18mL/plate of **Nutrient Agar + 3% w/v Tween 80**. Ensure plates are sufficiently full to allow a convex agar surface above the level of the plastic base.

- 3.3. Prepared Count-Tact plates will have an Expiry date printed on the surface of the lid. All other plates prepared within the Micro Lab have an Expiry date of 1 month from date of pouring. Store plates on compactor shelves.

3.3.1. Procedure for Sampling

- Record details of the Operator's name, clothing sampled, work area, BPN and date on the contact plate. **It is VERY important that all this information is written on the plates as they correspond to the batch and can affect batch release.**
- Decontaminate the plates by spraying with 70% IPA and place on the dividing bench in the **Sterile Scrub Preparation Room**.

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solution transfer 1mL onto a 150mm Lethen Agar plate. Using a sterile spreader, smear evenly over the agar surface. This is to be done in duplicate.

- 5.3. One plate is to be incubated at **32°C (± 1.5°C) for 72 hours (+24 hours)** and the other at **25°C (± 1.5°C) for (7days) 168 hours (+ 48 hours)**.
- 5.4. Limit: 10²cfu/ml of disinfectant solution.
(1 cfu plate is equivalent to 10 cfu in the original disinfectant sampled.)
- 5.5. The counts must be recorded in the Disinfectant Results File.
- 5.6. If the disinfectant is contaminated with 10 or more colonies on one plate, follow the action as outlined in section 9, 'OOL Disinfectants'.

6. Test Method for Swabbing

- 6.1. When conducting a swab of a surface area, use sterile swabs and sterile water for injection. Using aseptic technique, moisten the swab with the sterile water then swab over the test surface 5x5 cm. Spray swabbed surface with 70% IPA to remove any residue.
- 6.2. If a limit test is required place the swab inside a bottle of sterile Peptone water and snap off the stick without touching that part of the stick inside the bottle. Label the bottle with the location of the surface and the test date.
- 6.3. Before filtering shake well, under the laminar flow filter the contents of the bottle through a 0.45-micron membrane filter. Place the whole filter onto a sterile petri dish containing TSA. Incubate the dish at 32°C for 5 days. After incubation examine the petri dish and count the number of colonies present. Gram-stain and identify as per **MICLAB 070** Record all results in the Environmental Monitoring Comments Book.
- 6.4. If a Presence test is required place the swab directly into a 100ml bottle containing 50ml of TSB and break off the stick without touching that part of the stick inside the bottle. Incubate the bottle at 32°C for 5 days. Inspect daily. After the 5 days if no growth is apparent leave at room temperature for 7 days. If any growth is found, gram-stain and identify as per **MICLAB 070** Record all results in the Environmental Monitoring Comments Book.

7. Waste Tank Sampling for Sterile Filling Machines

- 7.1. It is the responsibility of the microbiology laboratory staff to organise sampling of waste tank waters by Sterile Operators or where are able themselves. Please see **MICLAB 055** for procedure.
- 7.2. Results are to be recorded on appropriate files.

8. Recommissioning of an Environmental Area

When an environmental area is to be returned to its correct Environmental Grade conduct an inspection audit of the area.

This will also include conducting environmental monitoring of the air and surfaces.

9. Out of Levels/Limits "OOL"

Alert Limits

If a test gives a one off result of equal to or above the Alert limit but less than the Action limit, a Deviation Report DR is not required. It must however be gram stained and future results for that location must be analysed to make sure it is not a chance of becoming an Action Limit risk. Record the investigation. Refer to each subsection on Air, Contact, Personnel, Settle plates and Disinfectants for required actions to take when the Alert limit is breached.

How and When to raise an Environmental DR

As stated in **Table A**, a DR is to be raised in the event of a result being equal to or above the **Action** limit, if the result has equalled or been above the Alert limit in 3 consecutive tests, or the result of a trend analysis over a period of one month shows that the mean value of monitoring results for an area exceeds the Alert Level. See also Tables B and C.

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- Where required, take the environment out of use immediately, until corrective action has been taken.
- Identify all organisms to at least Genus level.
- Over room pressure data for the room is to be reviewed.
- Area to be re-cleaned immediately and review the cleaning records for the past week.
- Review data from area for past 3 months.
- Visual inspection of the area for contributing factors, i.e. general cleanness level / adherence to procedures
- List of activities/ unusual processes that occurred on day of high monitoring results
- Check for any maintenance performed or due on equipment / system.
- Liaise with equipment / system owner to determine possible causes of OOL/OOS result.
- Hold investigation meeting to determine corrective and preventative actions, (CAPA's) and also determine impact on production activities.

9.4. OOL Surface / Swab Sampling

9.4.1. Alert Level

- Immediately re-sample.
- Report OOL result to manager.
- The organisms present are to be Gram stained and a record of this and their morphological appearance kept in the relevant forms.
- If any gram Negative rods are found, identify to Genus level.
- Perform three (3) days follow-up testing on point. **Note: If follow-up results indicate that the point is not under control the investigation should be elevated to an ACTION limit excursion.**

9.4.2. Action Level (in addition to Alert level requirements)

- Where required, take the environment out of use immediately, until corrective action has been taken.
- Identify all organisms to at least Genus level for sterile areas.
- Over pressure data for the room is to be reviewed
- Area to be re-cleaned immediately and review the cleaning records for the past week.
- Review data from area for past 3 months.
- Visual inspection of the area for contributing factors, i.e. general cleanness level /adherence to procedures.
- List of activities/unusual processes that occurred on day of high monitoring results.
- Check for any maintenance performed or due on equipment / system.
- Liaise with equipment / system owner to determine possible causes of OOL/OOS result.
- Hold investigation meeting to determine corrective and preventative actions, (CAPA's) and also determine impact on production activities.

9.5. OOL Personnel Monitoring

9.5.1. Alert Level

- Report OOL result to manager.
- Each morphological group present on the plate is to be Gram Stained. A record of this and their morphological appearance is to be kept in the relevant file.

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11. Summary Sheet - Recording Quick Reference

| Area | | Limit | | Procedure | |
|----------------------------|----------|-------|--------|---|--|
| | | Alert | Action | Alert | Action |
| <u>Sterile filling</u> | Air | <1 | <1 | Retest for 3 consecutive days, Gram stain, inform manager, analyse previous and following results. | Alert responses plus raise a DR . Notify Micro Manager/ Area Manager and/ or Service Engineers/ Cleaners. |
| | Surface | 2 | 5 | Retest for 3 consecutive days, Gram stain, inform manager, analyse previous and following results. | Alert responses plus raise a DR . Notify Micro Manager/ Area Manager and/ or Service Engineers/ Cleaners. |
| <u>Sterile preparation</u> | Air | 1 | 2 | Retest for 3 consecutive days, Gram stain, inform manager, analyse previous and following results. | Alert responses plus raise a DR . Notify Micro Manager/ Area Manager and/ or Service Engineers/ Cleaners. |
| | Surface | 3 | 5 | Retest for 3 consecutive days, Gram stain, inform manager, analyse previous and following results. | Alert responses plus raise a DR . Notify Micro Manager/ Area Manager and/ or Service Engineers/ Cleaners. |
| <u>Packing</u> | Air | 10 | 20 | Retest for 3 consecutive days, Gram stain, inform manager, analyse previous and following results. | Alert responses plus raise a DR . Notify Micro Manager/ Area Manager and/ or Service Engineers/ Cleaners. |
| | Surface | 12 | 25 | Retest for 3 consecutive days, Gram stain, inform manager, analyse previous and following results. | Alert responses plus raise a DR . Notify Micro Manager/ Area Manager and/ or Service Engineers/ Cleaners. |
| <u>Micro lab</u> | Air | 20 | 40 | Retest for 3 consecutive days, Gram stain, inform manager, analyse previous and following results. | Alert responses plus raise a DR . Notify Micro Manager/ Area Manager and/ or Service Engineers/ Cleaners. |
| <u>Personnel</u> | Gloves | 6 | 10 | Gram stain, inform manager, review operator's results. | Alert responses plus raise a DR to initiate an investigation. |
| | Uniforms | 3 | 5 | Gram stain, inform manager, review operator's results. | Alert responses plus raise a DR to initiate an investigation. |
| <u>Settle Plates</u> | Grade A | <1 | <1 | Gram stain, inform manager, review operator's results. | Alert responses plus raise a DR to initiate an investigation. |
| | Grade B | 3 | 5 | Gram stain, inform manager, review operator's results. | Alert responses plus raise a DR to initiate an investigation. |
| | Grade C | 25 | 50 | Gram stain, inform manager, review operator's results. | Alert responses plus raise a DR to initiate an investigation. |
| <u>Disinfectants</u> | | 10 | 20 | Retest , gram stain, inform manager, raise a DR to initiate an investigation. | |

12. Summary of Changes

| Version # | Revision History |
|------------|------------------|
| MICLAB 045 | New |

End of Procedure