

Standard Operating Procedure

Title: Environmental and Plant Hygiene Monitoring Procedure

Department	Micro Laboratory	Document no	MICLAB 045
Prepared by:		Date:	Supersedes:
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Approved by:		Date:	Review Date:

Document Owner

Micro Laboratory Manager

Affected Parties

All Microbiology Laboratory and production colleagues

Purpose

Description for Microbiological testing of areas of the environment which may influence or affect product performance and/or quality - including, air, surfaces, personnel, clothing and disinfectants.

Scope

This SOP is to be followed by all Microbiology Laboratory and production staff, in the execution of monitoring procedures outlined in this SOP.

Definition

Alert Levels	Alert Levels are accepted levels of contamination that have been derived statistically from "historical data", i.e. Levels that can be achieved under optimal operating conditions and the GMP guide lines.
Action Levels	Action Levels are twice the level of the Alert value or the officially stated maximum levels of contamination according GMP guide lines.
L/F	Laminar Flow
OOS	Out of Specification
OOL	Out of Levels/Limits
BPN	Batch Production Number
DR	Deviation Report
m/c	Machine
Genus	The first name of the scientific name (binomial); the taxon between family and species.
Species	The most specific level of taxonomic hierarchy.
TBA	To Be Advised
MLGEN	Microlab General area not classified
Settle Plate	Also called Fallout Plate

ALERT LEVELS and ACTION LEVELS MUST be clearly shown as appropriate on all graphical records held in the Environmental Test Reports Files .

Related Documents

Form 635	Daily Personnel Monitoring Log for Sterile Areas
MICLAB 005	Entry Procedure of Sterile Filling Area
MICLAB 010	Validation of Aseptic Gowning Procedures
QMS-035	Deviation Report System
MICLAB 055	Microbiological Monitoring of Plant Water Systems
MICLAB 015	Microbiological Data Recording Procedure
MICLAB 065	Determination of Heat Resistance of Spore Forming Organisms
MICLAB 060	Micro Laboratory Procedure for Sterility Testing

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of 1 (one) month shows that the <u>mean value</u> of monitoring results for an area exceeds the Alert Level.	Notify the Microbiology Manager /Area Manager and/or Service Engineers/Cleaners.
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An Environmental Monitoring Investigation form is to be the basis of the investigation but does not limit the scope of the investigation.

To facilitate final sign off of completed investigations the Investigation File is to be reviewed at 2 (two) monthly intervals by the Microbiology Manager.

OOL results will be reviewed in a two -phase approach. **Phase 1** is the review of the testing procedures by the microbiology technician to eliminate the possibility of lab introduced error or contamination. **Phase 2** investigations involve a thorough systematic analysis of the incident, to determine the root cause for the OOL result. Phase 2 investigations are to be tailored for each incident to reflect the significance of meeting or exceeding either the ALERT or ACTION limits as outlined in Table A.

1. Micro-Organism Levels in Air

- 1.1. Samples are to be taken using the MAS Air Sampling equipment, where organisms will become impinged onto **Trypticase Soy Agar**. When testing Laminar Flow make sure to turn on the L/F 10 minutes prior to sampling if not already on. Charge the Air Sampling equipment as required. The Air Sampling head must also be sterilized by autoclaving when the equipment is being recharged. Record details in the autoclave log and maintenance record book.
- 1.2. Prepared irradiated and non-irradiated **Trypticase Soy Agar (TSA)** plates purchased from an approved supplier are to be used in the monitoring of air inside the factory. Use irradiated plates in sterile areas and non-irradiated plates in lower environmental graded areas. If plates are not available prepare plates with approximately 25mL/plate of **Trypticase Soy Agar**. Ensure the petri dish has sufficient amount of agar to cover the surface of the entire plate and is not able to dry out.
- 1.3. Prepared TSA plates will have an expiry date printed on the surface of the lid. All other plates prepared within the microlab have an expiry date of 1 month from date of pouring. Store plates in refrigerator.
- 1.4. Each Agar plate should be labelled clearly with the date and the area sampled.
- 1.5. Plates are to be incubated at **32°C (± 1.5 °C) for 48 hours (+ 24 hours)**.
- 1.6. After each area is a sampled sign and date relevant form.
- 1.7. Record any unusual activities/observations about the area being monitored in the Environmental Monitoring Comments book, include date and time.
- 1.8. 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 Form is complete it must be signed by the Technician and reviewed by a Manager). After each area is a recorded sign and date relevant forms. Extended incubation is to be carried out for all areas sampled.
- 1.9. After the total count is recorded, re incubate the samples for a further **72 hours (+ 48 hours) at 25°C (± 1.5°C)**. Record the total count as well as number of moulds present on the histogram graph shading them in with a Green pen.
- 1.10. In the event that the result from the Air monitoring equals or exceeds either the ALERT or ACTION levels, follow the actions as listed under 'Micro Organisms in Air' see section 9.
- 1.11. **Sampling Areas:** Take one (1) sample from each area.

Summary: Table B for Incubation temperature , Incubation time and action .

Environmental Grade	Volume of Air Sampled	Incubation Time/Temp	Action Recording	Incubation Time/Temp	Action Recording
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Form is complete it must be signed off by the Technician and then reviewed by a Manager.

- 2.3.9. Sign and date the relevant form after each area is recorded. Extended incubation is to be carried out for all areas sampled.

After recording the total count, re-incubate the plates for a further 72 hours (+ 48 hours) at 25°C (± 1.5°C). Record the total count as well as number of moulds present on the histogram graphs shading them in with a Green pen.

- 2.3.10. In the event that the result from the Surface monitoring equals or exceeds either the ALERT or ACTION levels, follow the actions as listed under 'Micro Organisms on Surfaces', see section 9.

- 2.3.11. **Sampling Areas:** Take one (1) sample from each area.

Summary: Table C for Incubation temperature, Incubation time and action.

Environmental Grade	Incubation Time/Temp	Action Recording	Incubation Time/Temp	Action Recording
Sterile Filling	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.
Sterile preparation	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	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	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.
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- 2.3.12. **NOTE:** Contact Plate monitoring must also be conducted in all Sterile Filling rooms prior to or as close as possible to, the commencement of filling processes after any non-aseptic procedure has been carried out in the Sterile Filling Area, i.e. After "Open Day" maintenance, etc. **On these occasions the floor and walls are to be examined.** Alert Level for walls in the Sterile Filling areas = 2 colonies/plate.

Any deviation from this SOP is to be recorded in the "Environmental Monitoring Comments" book and a DR raised, if necessary.

3. Personnel

(See MICLAB 095 and MICLAB 060)

3.1. Operator's Gloved Hands

The number of viable micro-organisms on the gloved hands of Sterile Area Operators is to be enumerated daily for every batch using Fingerprint Impressions.

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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 (\pm 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 graphic ally 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 i s 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 o f 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

- a) 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 .**
- b) Decontaminate the plates by spraying with 70% IPA and place on the dividing bench in the **Sterile Scrub Preparation Room**.

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4.3. **Plates are incubated at 32°C (± 1.5°C) for 48 hours (+24 hours).**

Each plate is to be subjected to a total count and this information is to form part of the batch record. Results are to be recorded into the log book. This information forms part of the decision to release a batch for sale.

When recording the results in log book, comment the details of the fallout plate and who entered the details.

If any colonies are present record the morphological description and Gram stain reaction (microscopic appearance). Identify at least Genus Level. Record this information in the log book.

Action LIMIT:

Terminally Sterilised Product

Sterile filling <1 CFU/4 hours.

If any gpcr are present, D values must be determined. Limit for D -value is 1 minute for terminally sterilised products, (see **MICLAB 065 & MICLAB 075**).

Terminally Sterilised Product

Sterile filling 5 CFU/4 hours.

If any gpcr are present, D values must be determined. Limit for D -value is 1.5, (see **SOP MICLAB 065 & MICLAB 075**).

Action and Alert LIMIT

Aseptically Filled Product and Terminally Sterilised Product

Alert - 3 CFU/4 hours.

Action - 5 CFU/4 hours

In the event that the result from the Settle plate equals or exceeds either the ALERT or ACTION levels, follow the actions as listed under 'Micro Organisms on Settle Plates', see section 9.

5. Testing of Floor Disinfectant

5.1. The disinfectant used to clean the floors of the Sterile Area and the Factory must be tested on a weekly basis to ensure it is not contaminated with microbes. Sample into a sterile 20mL McCartney bottle at different sampling points.

5.2. Test Method

Table D

<u>Diluent</u>	<u>Disinfectant Group</u>
Nutrient broth	alcohols
	aldehydes
	hypochlorites
	phenolics
Peptone Water + 5% Tween 80	diguanides (at low concentrations)
	hypochlorites + detergent
	iodophors
	phenolics + detergent
	QUAT

Disinfectant Test Method:

A 1ml sample of the disinfectant solution is added to 9mL of a suitable diluent and mixed thoroughly. Allow standing time of 10mins. (This is to inactivate the disinfectant.) From this 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.

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Make sure that all required information is entered, i.e. Batch number, Author, Description and Department responsible.

What to add into the extended text box of the DR form

- Area
- Sample site
- Batch running at time of test (if applicable)
- Time
- Date
- Result
- Reason for the DR
- Person who took the sample
- Answers to as many Phase 1 Investigation questions as possible (see Section 9.1)

Example of Environmental Result DR

Sterile filler left side Air plate taken at 12pm dd/mm/yy running batch number XX XXXX, has a count of 2 colonies. This is above the Action Limit for this area as outlined in **MICLAB 045**. Mrs Y was the technician responsible for this test. She noted no unusual observations during the time the test took place. Collection, storage, preparation, set up of test equipment, incubation, calculation and test method procedures were followed correctly. Mrs Y's training records are complete and up to date. The media used was within expiry (TSA irradiated Batch number XXXXXX, expiry date dd/mm/yy) and media stasis and sterility were satisfactory. Gram Stain and ID work, if necessary, are pending.

Example of Personnel Monitoring DR

Mrs Y had a count of 6 colonies on her hood plate from the dd/mm/yy at 12pm. The batches running at the time of her being inside Sterile were XXXXXX, (add in all necessary BPN's). This is above the Action Limit for Uniform plates as outlined in **MICLAB 045**. She noted that there was maintenance being performed in a number of the rooms she entered. Collection, storage, preparation, set up of test equipment, incubation, calculation and test method procedures were followed correctly. Mrs Y's training records are complete and up to date. The media used was within expiry (Lethen Agar Batch number aaaaaa, expiry date dd/mm/yy, Count-Tact plates with Batch number bbbbbb, expiry dd/mm/yy) and media stasis and sterility were satisfactory. Gram Stain and ID work, if necessary, are pending.

Example of Settle Plate DR

Sterile filling Machine Left hand side Settle plate has a count of 6 cfu/4 hours from the dd/mm/yy, batch number XXXXXX. Start time: 10.30, Finish time: 14.30. This is above the Action level for this area according to **MICLAB 045**. Items such as set up of test equipment, test method, up to date training records and any observations made at the time of test are awaiting interview with the sterile operator responsible for running the test. Collection, storage, preparation, incubation and calculation procedures were followed correctly. Media used is within expiry (Nutrient agar batch number xxxxxx expiry dd/mm/yy) and media stasis and sterility were satisfactory. Gram Stain and ID work, if necessary, are pending.

Example of Disinfectant Sample Test DR

The Sterile filler disinfectant sampled on the dd/mm/yy by Mrs. Y and tested on the dd/mm/yy by Mr Z has a count of 21 colonies. This is above the Action limit for this area as outlined in **MICLAB 045**. Lisa noted no unusual observations during the time the test took place. Collection, storage, preparation, set up of test equipment, incubation, calculation and test method procedures were followed correctly. Mrs Y's training records are complete and up to date. The media used was within expiry (Lethen Agar Batch number xxxxxx, expiry date dd/mm/yy) and media stasis and sterility were satisfactory. Gram Stain and ID work, if necessary, are pending.

9.1. Phase 1 Investigation Responses

9.1.1. Phase 1 investigations for Environmental OOL results must consider, (but are not limited to), the following details:

- Correct collection, storage and preparation of test sample.
- Correct set-up of test equipment.
- Correct method used for test.
- Completed Training records.

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- 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.
- If any gram negative or positive rods are found, identify to Genus level.
- Ensure a review of the operator's results for the following 2 results.

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- Machine Cleaning records.
- Area to be re-cleaned immediately and review the cleaning records for the past week.
- Review all environmental data from the area, (i.e. personnel, air and surface).
- 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.
- Hold investigation meeting to determine corrective and preventative actions, (CAPA's) and also determine impact on production activities, batch disposition to be assessed.

9.7. OOL Disinfectants

In the event that the level has been reached, **Raise a Deviation Report** and the following Actions are to be considered as part of the OOL investigation:

- Immediately re-sample and test the sample point.
- Report OOL result to manager.
- Liaise with the cleaning co-ordinate for the extent of the use of the disinfectant, when manufactured, changed, expiry, correct storage and preparation procedures for the past week.
- Check when buckets last sterilised and have the buckets re-sterilised.
- Identify organism to at least Genus level. Results are to be recorded in the relevant files.
- If necessary, change the type of disinfectant being used in the area.
- Test the original concentrate.
- Perform a visual inspection of the storage area and preparation equipment and if necessary take swabs/samples.
- Review the microbiological water results used for the preparation of the disinfectant.
- Review Cleaning records for area.
- Hold investigation meeting to determine corrective and preventative actions, (CAPA 's) and also determine impact on production activities

10. Summary Sheet - Level of Identification

Area of Isolate	Colonial Morphology	Gram Stain	ID to Genus Level	Gram –ve rod ID
MONITORING -				
if over Alert Level:				
- Air Samples	X	X	x sterile	X
- Surfaces	X	X	x sterile	X
- Personnel Gloves	X	X	X if gpr/gnr	X
- Personnel Uniforms	X	X		
- Settle Plates	X	X	X	X
- Floor Disinfectants	X	X	X	X