

Standard Operating Procedure

Title: Microbiological Monitoring of Plant Water Systems

MICLAB 080	Bacterial Endo Toxin Testing (LAL) - Gel Clot Method
MICLAB 085	Bacterial Endo Toxin Testing kCA Method
MICLAB 110	Microbiology Laboratory Investigation and Retest Procedure for Atypical and OOS Results

EHS Statement

- Aseptic techniques should be used for all Microbiological procedures.
- Caution and care must be taken when sampling **hot** water from outlets.
- Safety (heatproof) gloves must be worn when sampling **hot** water.
- Earplugs must be used when sampling in Plant Water Room.
- Be careful when sampling in Services areas of overhead surface, pipes and air conditioning ductwork.
- **Safety Glasses and gloves** must be worn when using IPA or solvents.

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Procedure

1. General

- 1.1. Bioburden and Endotoxin testing is conducted on water collected from at least one sample point from the cold **WFI loop** each day, with each point of use tested at least weekly according to the schedule. All other grades / types of water are to be sampled and tested once per week.

2. Sampling Procedure for Bioburden and Endotoxin Samples

- 2.1. Bioburden and Endotoxin samples are to be collected at the same time. This ensures bioburden and endotoxin sample results can be compared.
- 2.2. Bioburden samples should be taken first.

2.3. Bioburden Sampling

- 2.3.1. Sterilised bottles should be labelled with the outlet point, the date and time the sample is collected.
- 2.3.2. Sampling must be conducted in employing good aseptic technique.
- 2.3.3. Spray inside and outside of sample port with 70% IPA. If a hose is attached to the outlet, sanitise the end of the hose using the same procedure.

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3.1.6. Incubate all plates inverted at between 30°C - 35°C for 5 days and examine and count the number of colonies present.

3.2. Results

2.3.1 Results and media batch details are to be entered into the log book. Enter the negative control result in the comments for each sample test.

2.3.2 Enter Time Tested under the Comments in the log book.

2.3.3 Growth on the sterile control is to be evaluated in the case where growth in a test sample meets or exceeds the alert limit.

2.3.4 When entering the results, the technician is to enter their signature (sign on) into the log book.

2.3.5 See section 5 for alert and action limits.

2.3.6 If any spore formers are isolated from a distilled water outlet, their heat resistance must be evaluated according to **MICLAB 065** The Alert Level for D-value of 1.5min.

2.3.7 Manager or appropriate delegate will prepare weekly reports for review to ensure all results have been reviewed and approved.

4. Endotoxin Testing of WFI (Distilled Water)

4.1. WFI is to be tested by either the LAL- Gel Clot Test Method (**MICLAB 080**) or the KCA Test Method (**MICLAB 085**)

4.2. WFI has an endotoxin Action limit of ≤ 0.25 EU/mL (the Action level). There is an Alert level of >0.125 EU/mL

5. Bioburden and Bacterial Endotoxin Alert and Action Levels

Water Type	Bioburden Testing	
	Alert Levels	Action Levels
Pre-Treatment Water	1000 cfu/ml	5000 cfu/ml
Chiller Water	100 cfu/mls (1000 cfu/10ml)	1000 cfu/mls (10,000 cfu/10ml)
Reverse Osmosis Water (RO)	10 cfu/ml (100 cfu/10ml)	100 cfu/ml (1000 cfu/10ml)
Purified Water (PW)	10 cfu/ml (100 cfu/10ml)	100 cfu/ml (1000 cfu/10ml)
Water for Injection (WFI)	1 cfu/100mls (5 cfu/500mls)	10 cfu/100mls (50 cfu/500mls)

Water Type	Endotoxin Testing	
	Alert Levels	Action Levels
Pre-Treatment Water	N/A	N/A
Chiller Water	N/A	N/A
Reverse Osmosis Water (RO)	N/A	N/A
Purified Water (PW)	N/A	N/A
Water for Injection (WFI)	0.125 EU/ml	0.25 EU/ml

5.1. In the event that the Alert or Action Level is exceeded for either Bioburden or Endotoxin results, initiate a laboratory investigation as per **MICLAB 110**.

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- 9.5. Conduct 3 consecutive repeat/follow up samples. In some cases due to a five-day incubation, follow-up sampling may occur as part of routine sampling. If this is the case then the collected sample results may be referred to as part of the repeat/ follow up investigation.
- 9.6. Upon completion of Phase 1 Investigation, if the OOS result is deemed to be valid raise a Deviation Report (DR) and refer to the investigation report.
- 9.7. A DR should also be raised if the following occur:
- Three or more sample points on the same ringmain on the same sampling date meet or exceed ALERT Level (Purified and WFI water only)
 - Three consecutive results from a sample point meet or exceed ALERT level.
 - The sample point meets or exceeds the ACTION level.
- 9.8. Under normal conditions Alert level excursions will only require organism identification, however action limit excursions require further investigation to determine a definitive cause and recommend corrective and preventative actions, (CAPA).

9.9. Summary Sheet - Recording Quick Reference

Water Type	Procedure	
	Alert	Action
<ul style="list-style-type: none"> • WFI 	<ul style="list-style-type: none"> • Initiate Lab investigation as per MICLAB 110. • Retest for 3 consecutive days, inform manager, • Analyse results of sample points on the same loop, if three or more points over Alert limit raise a DR. • Gram stain. Raise a DR if Gram Negative rods are detected and identify to species level. • Determine D-value of all spore-forming rods. 	<ul style="list-style-type: none"> • Alert responses plus raise a DR. • Notify Micro Manager/ Area Manager and/ or Service Engineers.
<ul style="list-style-type: none"> • Purified water • Chiller water 	<ul style="list-style-type: none"> • Retest for 3 consecutive days, Gram stain and identify any gram negative rods to species level. Inform manager. For purified water, analyse results of sample points on the same loop . • Additionally for Chiller water, contact engineering services and instruct to re-dose Chiller units with biocide. Commence follow-up sampling for 3 consecutive days following re-dosing. 	<ul style="list-style-type: none"> • Alert responses plus raise a DR. • Notify Micro Manager/ Area Manager and/ or Service Engineers.
<ul style="list-style-type: none"> • Pre-Treatment • RO water 	<ul style="list-style-type: none"> • Retest for 3 consecutive days, Gram stain isolates. Inform manager, analyse results of sample points on the same purified water system. 	<ul style="list-style-type: none"> • Alert responses plus raise a DR. • Notify Micro Manager/ Area Manager and/ or Service Engineers.
<ul style="list-style-type: none"> • Clean Steam 	<ul style="list-style-type: none"> • Retest for 3 consecutive days, Gram stain and identify any colonies to at least genus level and identify any gram-negative rods to species level. In the case that spore-forming organisms are isolated their heat resistance must be evaluated. Inform manager. 	<ul style="list-style-type: none"> • Alert responses plus raise a DR. • Notify Micro Manager/ Area Manager and/ or Service Engineers.
<ul style="list-style-type: none"> • Waste Water 	<ul style="list-style-type: none"> • Retest for 3 consecutive days, Gram stain, Gram negative rods need to be identified to species level, inform manager. 	<ul style="list-style-type: none"> • Alert responses plus raise a DR. • Notify Micro Manager/ Area Manager and/ or Service Engineers.

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11. Trending

- 11.1. Graphs are to be generated for WFI on a quarterly basis and for all other sample points on a 6 monthly basis.

12. Summary of Changes

Version #	Revision History
MICLAB 055	New

End of Procedure