

# 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.

### Table of Contents

1.	General .....	2
2.	Sampling Procedure for Bioburden and Endotoxin Samples .....	2
3.	Bioburden Testing .....	3
4.	Endotoxin Testing of WFI (Distilled Water) .....	4
5.	Bioburden and Bacterial Endotoxin Alert and Action Levels .....	4
6.	An example of Diagrammatic Representations of a typical purified Water Systems .....	5
7.	Bioburden Waste Tank Water Sampling .....	6
8.	Clean Steam Sampling & Testing .....	6
9.	OOL/OOS Result Actions .....	6
10.	Investigation task check list .....	8
11.	Trending .....	9
12.	Summary of Changes .....	9

### 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.

# Standard Operating Procedure

## Title: Microbiological Monitoring of Plant Water Systems

- 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  $\leq 0.25$  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](#).

# Standard Operating Procedure

## Title: Microbiological Monitoring of Plant Water Systems

- 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"> <li>• <b>WFI</b></li> </ul>	<ul style="list-style-type: none"> <li>• Initiate Lab investigation as per <b>MICLAB 110</b>.</li> <li>• <b>Retest</b> for 3 consecutive days, inform manager,</li> <li>• Analyse results of sample points on the same loop, if three or more points over Alert limit <b>raise a DR</b>.</li> <li>• Gram stain. <b>Raise a DR</b> if Gram Negative rods are detected and identify to species level.</li> <li>• Determine D-value of all spore-forming rods.</li> </ul>	<ul style="list-style-type: none"> <li>• Alert responses plus <b>raise a DR</b>.</li> <li>• Notify Micro Manager/ Area Manager and/ or Service Engineers.</li> </ul>
<ul style="list-style-type: none"> <li>• <b>Purified water</b></li> <li>• <b>Chiller water</b></li> </ul>	<ul style="list-style-type: none"> <li>• <b>Retest</b> 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 .</li> <li>• 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.</li> </ul>	<ul style="list-style-type: none"> <li>• Alert responses plus <b>raise a DR</b>.</li> <li>• Notify Micro Manager/ Area Manager and/ or Service Engineers.</li> </ul>
<ul style="list-style-type: none"> <li>• <b>Pre-Treatment</b></li> <li>• <b>RO water</b></li> </ul>	<ul style="list-style-type: none"> <li>• <b>Retest</b> for 3 consecutive days, Gram stain isolates. Inform manager, analyse results of sample points on the same purified water system.</li> </ul>	<ul style="list-style-type: none"> <li>• Alert responses plus <b>raise a DR</b>.</li> <li>• Notify Micro Manager/ Area Manager and/ or Service Engineers.</li> </ul>
<ul style="list-style-type: none"> <li>• <b>Clean Steam</b></li> </ul>	<ul style="list-style-type: none"> <li>• <b>Retest</b> 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.</li> </ul>	<ul style="list-style-type: none"> <li>• Alert responses plus <b>raise a DR</b>.</li> <li>• Notify Micro Manager/ Area Manager and/ or Service Engineers.</li> </ul>
<ul style="list-style-type: none"> <li>• <b>Waste Water</b></li> </ul>	<ul style="list-style-type: none"> <li>• <b>Retest</b> for 3 consecutive days, Gram stain, Gram negative rods need to be identified to species level, inform manager.</li> </ul>	<ul style="list-style-type: none"> <li>• Alert responses plus <b>raise a DR</b>.</li> <li>• Notify Micro Manager/ Area Manager and/ or Service Engineers.</li> </ul>

# Standard Operating Procedure

## Title: Microbiological Monitoring of Plant Water Systems

---

### 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*