GAP EnviroMicrobial Services Ltd.

APPROVAL FORM FOR RELEASE OF
ANALYTICAL STANDARD OPERATING PROCEDURE (SOP) FOR ROUTINE USE

SOP #90:
Legionella Sampling Instructions

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- **The approval of this document is valid for one year at which time it will be subject to review to determine if any updates or modifications are warranted.**

❖ *LEGIONELLA* SAMPLING INSTRUCTIONS

1. PURPOSE

1.1. This Standard Operating Procedure provides information on the proper collection of environmental samples during or following water system inspections to identify the potential presence of *Legionella* sources. The laboratory should be contacted to obtain sterilized sample containers and to arrange for transport and analysis **in advance.**

2. PROCEDURE

2.1. Determining Sample Strategy

Estimate the number of samples to be taken based on the size of the facility and the number of water systems indicated as potential *Legionella* sources. Sample sites that should be considered include:

2.1.1. Incoming Water Supplies, Storage Tanks and Hot Water Heaters

2.1.1.1. Collect samples aseptically from the bottom drains and outlet pipes of incoming water supplies, water storage tanks and hot water heaters.

2.1.2. Water faucets and Shower Heads

2.1.2.1. Water samples should be collected aseptically from both hot and cold-water faucets throughout the facility (i.e. nearest, intermediate and most distant from water heaters, storage tanks and municipal water supplies).

2.1.2.2. Collect the first water that leaves the faucet after the tap is opened.

2.1.2.3. Use sterile swabs to sample faucets and showerheads, and transport swabs submerged in sample water or existing neutralizing buffer.

2.1.3. Water-cooled Heat Exchangers.

2.1.3.1. Samples should be taken from the make-up water supply for the exchanger and for associated storage tanks and reservoirs. Collect additional samples at locations distant from the make-up water outlet and where water enters sprayers or misters. Include samples of sludge, sediment or biofilms, which can be taken by collecting liquid or using sterile swabs.

2.1.4. Humidifiers, Spas, Decorative Fountains and other equipment.

2.1.4.1. Collect water from tanks and reservoirs, as well as from supply water for comparison. Include swab samples of sediment or biofilm if present.

Table 1. Miscellaneous Sampling Sites

Tepid-water eye wash stations	Spray-cooled cutting machines
Safety showers	Molding presses
Fruit & vegetable misters	Pasteurizers
Spray irrigation systems	Roof sprays for humidity control
Fire sprinkler storage tanks	

2.2. Sample Collection Procedure

2.2.1. Collect 1 L of water for culturable analysis or 500mL of water for PCR analysis from cooling towers and other water-cooled heat exchange systems. Use clean, sterile containers with neutralizing agent provided by our laboratory. Sodium thiosulphate, a neutralizing agent, is added into the sample bottles provided by the laboratory. The bottles contain sufficient neutralizing agent to remove chlorine at concentrations used in most water storage facilities.

2.2.2. Use aseptic sampling procedures. Sample containers should remain closed until just before the sample is taken, and closed again immediately after sampling. The 1L sample bottles provided by the laboratory should be filled to the top of the label, to allow mixing of the sample at the laboratory.

2.2.3. Sterile swabs can also be used to collect samples, and can be obtained from our laboratory. Use the swab to remove material from the suspect site, and replace the swab into the container provided. Using water from the same sampling location, fill the container half full to keep the swab wet. Do not use chlorinated tap water for this. Otherwise, replace swab in supplied neutralizing buffer.

2.2.4. Carefully label each sample container, and include a fully completed GAP chain of custody with each submission. Chain of custody forms can be obtained by contacting the laboratory, or are available from the GAP website (www.gapenviromic.com).

2.2.5. Samples must be kept cool (not frozen), and protected from temperature extremes during storage and transport. Samples should reach the laboratory within 48 hours of sampling.

2.3. Personal Protection During Sampling

2.3.1. Request that equipment be turned off while collecting samples, where possible. Wear disposable garments, slip-proof footwear and eye protection while working in areas that are wet, potentially contaminated, or recently treated with biocides/disinfectants. Wear at least a 95% efficient, half-mask respirator when working near potentially contaminated equipment that might generate aerosols.

3. HISTORY OF CHANGES:

3.1. Revision 1 – May 1, 2006

3.1.1. New

3.2 Revision 2 - October 10, 2007

3.2.1 This SOP was revised to reflect the company name change.

3.3 Revision 3 – July 15, 2008

3.3.1 Revision 3 was reviewed and no changes were required.

3.4 Revision 4 – April 23, 2013

3.4.1 Section 2.2.1 was updated to include the sample requirements for PCR analysis.

4. REFERENCE:

4.1. ASTM Method D 5952 (Inspecting Water Systems for *Legionellae*).

4.2. Resources for Sampling:

http://www.osha.gov/dts/osta/otm/legionnaires/cool_evap.html

http://www.osha.gov/dts/osta/otm/legionnaires/sampling.html

http://www.osha-slc.gov/dts/osta/otm/otm_iii/otm_iii_7.html