Bio Lp-1 DNA isolation kit

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1. INTRODUCTION

Legionnaires Disease (LD) is a severe and often fatal form of pneumonia which is caused by bacteria known as Legionella. It is predominantly transmitted through contaminated water systems. Older adults, smokers and immunocompromised patients are particularly susceptible to LD. Globally the incidence of LD is estimated at 180–360 cases per million inhabitants, with the rate of incidence increasing in many parts of the world[1]. Infections acquired in buildings and healthcare institutions affect approximately two million people each year resulting in 90,000 deaths and an estimated $4.5–5.7 billion per year in additional costs for patient care[2]. Current European estimates attribute an additional 16 million days spent in hospital by patients per year[3]. Within these settings, water, water distribution and premise plumbing systems have been identified as a significant source of many of Health Care Associated Infections (HCAI) and pose a significant threat to human health.

The most effective way to limit the spread of LD is to control and eliminate the bacteria from environmental reservoirs. At present the most commonly used LD tests for water rely on century old culture methodologies which require significant hands-on time, are time consuming, slow (>14 days to result) and lack specificity and sensitivity.

An alternative approach is to apply molecular based LD water testing technologies which have the capability to provide rapid (< one day) and robust results. Such approaches can be highly specific for Legionella, while also being very sensitive. Any such test should be capable of detecting all Legionella species associated with infection, while also specifically identifying all serogroups of Legionella (16 serogroups responsible for causing majority of disease) and ideally also identifying *L. pneumophila* serogroup 1 which is reported to cause ~95% of human infections.

BioProbe Diagnostics have developed a new molecular kit called Bio Lp-1 which offers advantages over the most commonly used traditional testing methods in terms of time to result, specificity and sensitivity. Bio Lp-1 provides a complete identification of Legionella to the serogroup level in a single test using molecular technologies. Bio Lp-1 is the first combined test with a capability for the rapid (< 5 h from sample in to result out), sensitive, quantitative detection and identification of Legionella, *L. pneumophila* and *L. pneumophila* sg1 in a water sample.

[1] https://www.cdc.gov/legionella/surv-reporting.html

[2] Collins AS. Preventing Health Care–Associated Infections. 2008 Apr. Chapter 41.

[3] European Centre for Disease Prevention and Control: Annual Epidemiological Report on Communicable Diseases in Europe 2008

2. PRODUCT DESCRIPTION

- Bio Lp-1 provides a simple procedure for fast optimal total DNA extraction and purification from water samples.
- High-quality DNA is purified and eluted with Elution Buffer. Purified DNA can be used for downstream applications such as real-time PCR.
- Recovery of genomic DNA up to 40 kb.

3. TEST PRINCIPLE

Sampling, transport and storage of water samples should be performed according to ISO 19458. Water samples are concentrated by filtration. DNA extraction is performed directly from the filter yielding high quality DNA for use in downstream applications such as PCR.

4. KIT CONTENTS

DNA Extraction and Purification	**50 preps**	**Kit Storage Temperature**
Genomic lysis buffer	50 ml	Room Temperature
DNA pre-wash buffer**	15 ml	Room Temperature
Wash buffer	50 ml	Room Temperature
Elution buffer	10 ml	Room Temperature
Filter spin columns	50	Room Temperature
Collection tubes	100	Room Temperature

** A precipitate may form in the DNA pre-wash buffer during shipping or storage. To dissolve the precipitate, incubate the buffer at 37 ˚C for 30 minutes and mix gently by inversion. DO NOT MICROWAVE SOLUTION

5. KIT STORAGE AND SHELF LIFE

All kit contents should be stored at room temperature. Each reagent stored at this temperature may be used until the expiration date indicated. Do not freeze the reagents from the kit.

6. EQUIPMENT AND MATERIALS REQUIRED BUT NOT SUPPLIED

6.1. Equipment

Water filtration

- Filtration apparatus (mounted either on air pump or on vacuum flask) in a Legionella free environment.
- Sterile filter funnel
- Biological safety cabinet (BSC II) / Legionella free area

DNA Extraction and Purification

- Biological safety cabinet (BSC II) / Legionella free area
- Rolling/rocking platform
- Micro-centrifuge with a rotation capacity of at least 10,000 x g

6.2. Laboratory Supplies

Water filtration

- Sterile forceps
- Membrane filter (recommended membrane filter – polyethersulphone (PES) filter with pore size of 0.2 - 0.45 µm (Recommended filters PALL PES 0.45 µm). A preliminary validation should be performed in the laboratory if any other types of membrane filters are used. Membrane filters containing cellulose are not recommended.
- 5 ml screw cap, conical bottom tubes to transfer membrane filters after water filtration (such as Sarstedt 5 ml conical bottom tubes).

DNA Extraction and Purification

- Micropipettes 100-1000 µl, 20-200 µl and 2-20 µl
- Filtered pipette tips to fit above micropipettes
- 1.5 ml microcentrifuge tubes (sterile, DNase, RNase and pyrogen free)

Other Consumables needed

- PPE mask
- Unpowdered gloves
- Decontamination solution e.g. 70% alcohol or 5% sodium hypochlorite solution

7. WATER SAMPLING AND SAMPLE TRANSPORTATION

Samples are collected according to the general standards for water quality analysis and bacteria detection and enumeration in accordance with ISO 19458 and ISO/TS 12869. Samples should be collected in clean, sterile containers with all necessary precautions.

The samples should be processed as soon as possible after sampling. If the samples are delivered for analysis within 24 h, they can be shipped at room temperature.
If the time between sampling and arrival at the laboratory for analysis is greater than 24 h samples should be shipped and stored at +5 °C ± 3 °C. The storage of the sample for longer periods or at different temperatures must be validated.
Water samples treated with oxidizing biocide must be treated with a sufficient quantity of sterile inactivation agent such as sodium thiosulfate. If water is treated with other biocides, a suitable neutraliser must be used, if available. In the case of a biocide that cannot be neutralised, their presence must be declared and indicated on the test report.

8. WATER FILTRATION PROTOCOL

i. Place a 0.2 - 0.45 µm membrane filter on the sterile filtration apparatus. (For more turbid samples see ISO 19458)
ii. Fit the sterile funnel to the apparatus.
iii. Filter 100 mL to 1 L of water through the membrane filter (recommended volume 1L).
iv. Single use sterile filter funnels are recommended. However, if reusable filter funnels are used, ensure the funnel and manifold are appropriately decontaminated between each water sample. For example, rinse the filtration manifold with at least 100 ml of Legionella DNA free water and then decontaminate with alcohol after filtration of each water sample to avoid crossover DNA or bacterial contamination.
v. Repeat as necessary for each water sample.

9. DNA EXTRACTION AND PURIFICATION PROTOCOL

i. Using sterile forceps, carefully place the membrane filter in sterile 5 ml collection tube (not provided) with the filtrate facing the centre of the tube.
ii. Add 900 µl genomic lysis buffer to tube containing membrane filter. Ensure filter is coated with lysis buffer. Place tube on rolling platform for 10 min and ensure filter is repeatedly washed with the lysis buffer (50-100 rpm).
iii. Transfer 800µl of lysate to a spin column placed in a collection tube. The membrane filter should be discarded appropriately. Centrifuge the spin column at 10,000 x g for 1 min. Discard the collection tube with the flow through.
iv. Transfer the spin column to a new collection tube. Add 200 µl DNA Pre-wash Buffer. Centrifuge at 10,000 x g for 1 min.
v. Add 500 µl DNA wash buffer to the spin column. Centrifuge at 10,000 x g for 1 min. Discard the collection tube with the flow through.
vi. Transfer the spin column to a sterile microcentrifuge tube. Add 100 µl DNA elution buffer to the spin column.

vii. Incubate at room temperature for at least 2 min and then centrifuge at top speed for 30 sec to elute the DNA. Note: In this step, the cap of the microcentrifuge tube cannot be closed.

viii. For maximum recovery from filter column, recover the eluant and add to the column a second time, repeating step vii.

ix. The DNA can be used immediately in the Bio Lp-1 Legionella Detection Kit (BPD 2001) or the Bio Lp-1 Legionella Quantification Kit (BPD 3001). It is recommended that the DNA is used immediately for downstream applications. If storage is required, it is recommended that the DNA is stored at 4 ˚C for no more than 24 h. If longer storage or freezing of the samples is required, this method must be validated by the testing laboratory.

10. PRECAUTIONS

IMPORTANT: It is essential that this Protocol be carried out by suitably trained staff.

- Do not use reagents after the kit expiry date.
- Ensure all work surfaces are regularly decontaminated using suitable solution e.g. 5% sodium hypochlorite solution or 70% alcohol.
- Gloves should be worn throughout the procedure. Gloves should be changed regularly and after suspected contact with contaminants.
- Laboratory consumables should be sterile, DNase and RNase free.
- Laboratory equipment should be appropriately decontaminated before each use.
- All water samples containing unknown microorganisms should be handled and disposed of as potentially infectious substances.
- Persons using this Protocol should be familiar with good laboratory practice.

11. TROUBLESHOOTING

Degradation of DNA after purification

All supplied reagents and consumables are DNase free. However, DNase contamination may be introduced during the DNA extraction and purification process of certain samples. Ensure that all pipette tips and tubes used for sample processing are DNase free. Appropriate precautions during the procedure should safeguard against DNase contamination.

Downstream applications using purified DNA are not performing as expected

Check that the correct volume of Lysis Buffer has been added to each sample. Confirm that all samples were incubated for the specified time. Failure to perform the above steps correctly may result in insufficient lysis of the bacterial cells.

Subsequently, ensure that all centrifugation steps are completed at the speeds and times indicated in the protocol. If these steps are not performed correctly the sample may not be washed completely and some salts may be retained on the filter column and eluted along with the DNA. This may have an adverse effect on downstream applications such as DNA quantification and real-time PCR.

VALIDATION

Bio Lp-1 DNA ISOLATION KIT is certified by MicroVal according to a validation protocol based on ISO 12869:2019, Water quality - Detection and quantification of Legionella spp. and/or Legionella.
pneumophila by concentration and genic amplification by quantitative polymerase chain reaction (qPCR).
MicroVal certification No. xxx
Valid until __/__/____