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VAT Reg No: 1623-1407

Method statement

Ref: MS-MNA-230222-Discharge point sampling

Date: 23 April 2024

Client: Marine Aquatic Limited

Re: Monitoring programme in relation to the environmental permit held by the Marine Aquatic Limited for its discharge to sea

Assignment details

The present submission has been prepared in response to a request by Marine Aquatic Limited, whereby Ecoserv Ltd. (hereafter Ecoserv), have been requested to submit a proposal for methodology the Improvement Programme outlined in the environmental permit (hereafter 'Permit'), held by Marine Aquatic Limited, for its discharge to sea from emission points ED1: Exhibit and Quarantine livestock tanks. Request states the following:

To submit a revised monitoring proposal for water quality monitoring of the effluent prior to discharge to sea well ED1 aimed at determining the physico-chemical, microbial and nutrient load of the effluent in line with Table 2.1.3. The monitoring proposal shall also include: Detection limits and standards used for each parameter

- *Sampling methodology*

Proposal

Most of the parameters that would adequately assess the "physico-chemical, microbial and nutrient load of the effluent" are already being monitored as part of the annual environmental report (AER), that is based on three monitoring sessions per year that take place during peak summer, summer, and winter. The list of parameters that are already monitored thrice yearly at ED1 is given along with the requested limits and laboratory limits of detection (LoD) in Table 1. Given that this list is already fairly extensive, Ecoserv is proposing to use the same list of parameters for the improvement programme, with the addition of some further parameters that are deemed relevant. The proposed parameters are Total Organic Carbon (TOC), Total Dissolved Solids (TDS) and microbiology tests (*E. coli* and Faecal Enterococci) related to bathing water quality, as the effluent to the sea is located not far off from a designated bathing area.

Sampling for microbiological tests will follow the ISO 19458 method. The results of microbiological tests will be compared to the threshold values for coastal waters specified in the EC's Directive 2006/7/EC concerning bathing water quality. It should be noted that the

thresholds stipulated in Directive 2006/7/EC are used to assess the quality of bathing water, not the discharge water, given that there is a high degree of dilution in the former. Comparisons to these thresholds should therefore be made with a degree of caution.

Ecoserv proposes that collection of samples to test these parameters from ED1 will be made at the same frequency as sampling for the AER; i.e. thrice yearly. Sampling for AER parameters and the Improvement Programme can take place during the same sampling session, such that the results generated from a single sampling session can be utilised to populate the AER, and also for the Improvement Programme, seeing that a number of the parameters are common to both lists. The extended list of parameters, that would include analysis of samples for both the AER and for the improvement programme, is outlined in Table 2.

Table 1 – Currently monitored parameters at ED1, from which samples are collected three times a year to compile the AER.

Emission point	Parameter	Limit	In-situ / Laboratory Analysis	Instrument / Laboratory LOD
ED1 Exhibit and Quarantine livestock tanks	pH	6 - 10	In-situ	0.01
	Salinity	n/a	In-situ	0.01 ppt
	Temperature	40°C or 5°C above ambient, whichever lowest	In-situ	0.1°C
	Total Nitrogen	10 mg/L	Laboratory	0.1 mg/l
	Ammonium	n/a	Laboratory	15 µg/l
	Orthophosphates	n/a	Laboratory	62 µg/l
	Total Phosphorous	1 mg/l	Laboratory	20 µg/l
	COD	125 mg/l	Laboratory	2 mg/l
	BOD	25 mg/l	Laboratory	1 mg/l
	TSS	35 mg/l	Laboratory	2 mg/l

Frequency of monitoring

Monitoring will be carried out three times yearly during peak summer, summer, and winter, as for the other parameters in the AER.

Personnel

Supervision and coordination of all work and analyses of data, and writing of the report will be carried out by Professor Joseph A Borg BSc MSc PhD (Plymouth) CBiol MRSB MMBA FIBMS, assisted by a team of environmental biologists from Ecoserv led by Mr Andrew Agius BSc (Hons).

Delivery

4 – 6 weeks from date of commissioning.

Requirements

Free and unhindered access to the site.

Table 2 – Proposed extended list of parameters at E1, from which samples would be collected three times a year to compile the AER, and to satisfy the requirements of the Improvement Programme, Item 9a.

Emission point	Parameter	Limit	In-situ / Laboratory Analysis	Instrument / Laboratory LOD	Analytical method
ED1 Results to be used for both AER and Item 9a	pH	6 - 10	In-situ	0.01	In-situ meter
	Salinity	n/a	In-situ	0.01 ppt	In-situ meter
	Temperature	40°C or 5°C above ambient, whichever lowest	In-situ	0.1°C	In-situ meter
	Total Nitrogen	10 mg/L	Laboratory	0.1 mg/l	Digestion
	Ammonium	n/a	Laboratory	15 µg/l	Discrete analyser
	Orthophosphates	n/a	Laboratory	62 µg/l	Discrete analyser
	Total Phosphorous	1 mg/l	Laboratory	20 µg/l	ICP-OES
	COD	125 mg/l	Laboratory	2 mg/l	Colorimetric
	BOD	25 mg/l	Laboratory	1 mg/l	BOD probe
	TSS	35 mg/l	Laboratory	2 mg/l	Gravimetric
Results to be used for Item 9a only	TDS	n/a	In-situ	1 mg/l	In-situ metre
	TOC	n/a	Laboratory	0.1 mg/l	TOC analyser
	<i>E. coli</i>	Refer to Directive 2006/7/EC	Laboratory	1 cfu/100ml	Membrane filtration followed by culture
	Faecal enterococci		Laboratory	1 cfu/100ml	Membrane filtration followed by culture

Annex 1

Accreditation certificate of:

- **I2A Laboratories**
- **BioDNA**

Certificate of Accreditation



UKAS
United Kingdom
Accreditation
Service

I2 Analytical Ltd

Testing Laboratory No. 4041

**Is accredited in accordance with International Standard ISO/IEC 17025:2017
– General Requirements for the competence of testing and calibration
laboratories.**

This accreditation demonstrates technical competence for a defined scope specified in the schedule to this certificate, and the operation of a management system (refer joint ISO-ILAC-IAF Communiqué dated April 2017). The schedule to this certificate is an essential accreditation document and from time to time may be revised and reissued.

The most recent issue of the schedule of accreditation, which bears the same accreditation number as this certificate, is available from www.ukas.com.

This accreditation is subject to continuing conformity with United Kingdom Accreditation Service requirements.



Matt Gantley, Chief Executive Officer
United Kingdom Accreditation Service

Initial Accreditation: 6 July 2006
Certificate Issued: 25 January 2021



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UKAS is appointed as the sole national accreditation body for the UK by The Accreditation Regulations 2009 (SI No 3155/2009) and operates under a Memorandum of Understanding (MoU) with the Department for Business, Energy and Industrial Strategy (BEIS).



BioDNA Laboratory Services Ltd

Scope of Accreditation

Contact person	Marisa Cassar
Address	BioDNA Laboratory Services Ltd., LS2.1.1 and LS2.1.2, Malta Life Sciences Park, San Gwann Industrial Estate, San Gwann, SGN 3000, Malta
Telephone	+356 21665164
Company Reg. No.	C37173
Email	contact@biodna.net
Website	www.biodna.net

ACCREDITATION INFORMATION - TESTING LABORATORY

Accreditation No.	005
Accreditation Certificate No.	005/15
Accredited according to	EN ISO/IEC 17025:2017
Accreditation Scope No.	S005/15
Date of issue of this Scope	Friday, 22 September 2023

SCOPE OF ACCREDITATION

Issue No: S005/15

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TESTING LABORATORY

Laboratory Locations

Location Details	Activity	Location Code
Address BioDNA Laboratory Services Ltd., LS2.1.1 and LS2.1.2, Malta Life Sciences Park, San Gwann Industrial Estate, San Gwann, SGN 3000, Malta	DNA Tests Forensic Analysis Food and Water Microbiology Analysis Drugs of Abuse Analysis	A

Site activities performed away from the locations listed above

Location Details	Activity	Location Code
Clients	Sampling for subsequent microbiological testing, DNA analysis, Drugs of Abuse Analysis	B

NAB-Malta is a signatory for the EA MLA in testing, calibration and inspection

National Accreditation Board - Malta (NAB - MALTA)
 Mizzi House, 1st Floor, National Road, Blata l-Bajda HMR9010, Malta
 Tel No. (+356) 23952510
 Web: www.nabmalta.org.mt - Email: info@nabmalta.org.mt

 ISO/IEC 17025 ACCREDITED TESTING N° 005	BioDNA Laboratory Services Ltd Scope of Accreditation
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SCOPE OF ACCREDITATION	S005/15	issued on 22/09/2023	Page 2 of 6
Material/ Product/ Matrix Tested	Type of test, parameter/ component/ characteristic measured, range of measurement, equipment	Standard Specifications/ In-House Methods /Techniques	Loc. code

Body Fluid Identification

Any material or object	-Forensic stain search on exhibits -Presumptive testing for presence of blood and semen	In house method using tests for enzyme activity: - MBDSOPTC019 - MBDSOPTC020 - MBDSOPTC091	A
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DNA Analysis

Extracted DNA	Forensic Analysis DNA profiling: - Analysis of STR loci using ABI3500 Genetic Analyzer - Analysis of STR loci using Investigator 24Plex QS kit and ABI3500 Genetic Analyzer - Comparison of forensic DNA results within the case	In house methods using DNA amplification (PCR), electrophoresis and genetic characterization. - MBDSOPTC0118	A
Extracted DNA	Paternity and relationship testing using Investigator 24Plex QS kit and ABI3500 Genetic Analyzer	In house methods using DNA amplification (PCR), electrophoresis and genetic characterization, - MBDSOPTC017 - MBDSOPTC018	A
Extracted DNA	Quantification of Human DNA using the ABI7300 and Quantifiler Human DNA Quantification kit	MBDSOPTC119	A

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Material/ Product/ Matrix Tested	Type of test, parameter/ component/ characteristic measured, range of measurement, equipment	Standard Specifications/ In-House Methods /Techniques	Loc. code
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Swabs including Buccal Swabs from any material or object	DNA extraction manual methods using Genomic DNA extraction Kit, Accuprep kit	MBDSOPTC0120	A
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Buccal swab	Sample Collection	MBDSOPTC043	A
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Drugs of Abuse Analysis

Drugs	Detection and identification of: - Cocaine (1%) - Heroin (1%) - THC (Delta-9 tetrahydrocannabinol) (1%) - MDMA (3,4-Methylenedioxymethylamphetamine) (1%)	MBDSOPTC0126 using GC-MS	A
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Drugs	Quantification of: - Cocaine (2%) - Heroin (2%) - THC (Delta-9 tetrahydrocannabinol) (2%) - MDMA (3,4-Methylenedioxymethylamphetamine) (2%)	MBDSOPTC0126 using GC-MS	A
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Microbiology

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Material/ Product/ Matrix Tested	Type of test, parameter/ component/ characteristic measured, range of measurement, equipment	Standard Specifications/ In-House Methods /Techniques	Loc. code		
Food products	Enumeration of Staphylococcus aureus (confirmation by latex test)	MBDSOPTC031	A		
Food products	Enumeration of total coliforms	EN ISO 4832:2006	A		
Food products	Aerobic colony count at 30°C by the pour plate technique	EN ISO 4833-1:2013 / AMD 1:2022	A		
Food products	Detection of Salmonella spp.	EN ISO 6579-1:2017/Amd 1:2020	A		
Food products	Enumeration of Escherichia coli – colony count technique at 44°C	ISO 16649-2:2001	A		
WATERS: groundwater, drinking water, recreational water, saline water, process water.	Detection and enumeration of Legionella	ISO 11731:2017, Matrix A & B, Procedures 5 & 9 (including Annex F)	A		

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Material/ Product/ Matrix Tested	Type of test, parameter/ component/ characteristic measured, range of measurement, equipment		Standard Specifications/ In-House Methods /Techniques		Loc. code		
WATERS: groundwater, drinking water, recreational water, saline water, process water	Total count of colonies formed in a nutrient agar culture medium after aerobic incubation at 22°C and 37°C		ISO 6222:1999		A		
WATERS: groundwater, drinking water, recreational water, saline water, process water	Detection and enumeration of <i>Pseudomonas aeruginosa</i> – method by membrane filtration		EN ISO 16266:2006		A		
WATERS: groundwater, drinking water, recreational water, saline water, process water	Enumeration of <i>Escherichia coli</i> and coliform bacteria – membrane filtration method for waters with low bacterial background flora		EN ISO 9308-1:2014/Amd 1:2016		A		
WATERS: groundwater, drinking water, recreational water, saline water, process water	Detection and enumeration of intestinal enterococci – membrane filtration method		EN ISO 7899-2:2000		A		
WATERS: process water	Sample collection for microbiological analysis		EN ISO 19458:2006		B		
Fungal and Yeast identification	Identification of yeasts and moulds by ITS region by PCR		Internal SOP - MBDASTC0068, MBDASTC037		A		

 ISO/IEC 17025 ACCREDITED TESTING N° 005	BioDNA Laboratory Services Ltd Scope of Accreditation
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Material/ Product/ Matrix Tested	Type of test, parameter/ component/ characteristic measured, range of measurement, equipment	Standard Specifications/ In-House Methods /Techniques	Loc. code		

Bacterial identification

Identification of bacteria by
16s rRNA PCRInternal SOP -
MBDASTC0057,
MBDASTC037

A

END OF SCOPE

This scope of accreditation may be revised from time to time by NAB-MALTA. The most recent version of this scope may be found from the NAB-MALTA website. Nevertheless, as technical issues may hinder the immediate update of the website, and in case of any difficulty, contact the NAB-MALTA on +356 23952510 or by sending an email to 'info@nabmalta.org.mt'.

NAB-MALTA