AK 026

Statement of Verification

EU Environmental Technology Verification Programme

Technology Type:	**Monitoring Technology**
Technology Name:	**aquaBio B403**
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THE VERIFICATION PROCESS, WHOSE RESULTS ARE SUMMARISED IN THIS STATEMENT, COMPLIES WITH THE EU-ETV GENERAL VERIFICATION PROTOCOL AND WITH THE ISO STANDARD 14034 ON ENVIRONMENTAL MANAGEMENT: ENVIRONMENTAL TECHNOLOGY VERIFICATION.

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Internet address where the Statement of Verification is available:
https://circabc.europa.eu/ui/group/cd5138da-9303-4dec-b82c-bb29409ecdbd/library/485ebc03-6456-4310-9521-65759d3cb90b

1 Technology description

aquaBio B403 is an on-line system for detection and quantification of E. coli in water on a continuous basis. The operation of the equipment is completely autonomous and requires only electric power supply, sample input, drainage, disinfectant for disinfecting the equipment and the specific reagent.

Figure 1 General view of aquaBio 403

The system is composed of an hydraulic circuit, an electrical circuit, electronics and software, all of them managed by the programmable logic controller PLC.

The hydraulic system configuration together with the software operation guarantees the independence between samples by operating the different elements.

Electrical system

The electrical system is composed of PLC controller and Multiparameter. The Multiparameter unit converts the optical signals into electric signals that are then transduced, adapted, collected and interpreted by the PLC. The PLC controls the process, activation of valves and pumps and the electrical circuit that connects the electronics with the physical elements. The PLC, as a datalogger, stores up to 100 results. The results can either be viewed on a display (HMI) of the instrument or downloaded locally or remotely to a PC or to a control center described further in the Communication section.

Communications

In the front part of the equipment (Figure 1) there is a touchable screen from where all the manual operations are made and the results are displayed. aquaBio B403 is flexible as well regarding the Communications. The different options are:

- a Digital Output (DO): When a threshold is exceeded it activates a DO,
- a Digital input (DI): To stop; Force a disinfection, force an analysis,
- local USB excel,
- serial port communications (RS232, RS485) through MODBUS RTU: Status, Events/Alarms, Measures,
- ethernet through:
    - WEB server -> Download historical values events and alarms. Firmware update. Obtention of an Excel file.
    - ecoData (MQTT protocol) -> Instant communication with IoT data viewers. Transmission of values, events and alarms. (ecoData Viewer).
- The principle of aquaBio B403 operation is based on fluorescence detection method using defined β– Glucuronidase substrate positive by E. coli and on colour detection method using β-galactosidase substrate positive by coliform bacteria, that are correlated to the concentration of bacteria in liquid medium calculated as Most Probable Number (MPN). aquaBio B403 automatically takes 100 ml of water sample according to the programmed intervals. The sample is introduced into the measurement chamber together with the reagent that contains 4-methylumbeilliferyl-beta-D-glucuronide (MUG) and o-nitrophenyl-beta-D-galactopyranoside (ONPG)), substrates for enzymatic reactions. When the substrate is degraded by the bacteria producing colour (coliform bacteria) and fluorescence (E. coli) the optical system starts measuring the absorbance and the fluorescence of the sample every 2 min. The time of appearance of colour or fluorescence is correlated to the concentration that is calculated by the system as MPN. The measurement of samples is estimated between 5 and 13,5 hours to complete each assay, including the time of warming, cleaning, taking the sample and performing the analysis.
- aquaBio B403 can operate in different modes: diary, continuous, by an external alarm or by episode, giving a large flexibility to the operator to run the equipment depending on their needs. The device can act as an autonomous station controlling an external pump for recirculation or on external elements thanks to the digital outputs.

The communications developed in the equipment allow to act locally through Human-Machine Interface (HMI) or remotely through a control centre. The ecoData (web service developed by ADASA SISTEMAS S.A.U.) can collect the data from the aquaBio B403, represent them, consult the proper functioning of the equipment, and notify the alarms generated in case of exceedance of the configurated thresholds.

aquaBio B403 can be used as an Early Warning System in various applications e.g. for detecting malfunction of a wastewater treatment plant or other functions with focus on water safety and minimizing pathogenic risks associated with the reuse of water. aquaBio B403 may provide wastewater treatment operators/ administrators with current data necessary for:

- proper operation of a WWTP in terms of microbiological quality of effluent,
- early warning in case of the WWTP malfunctioning in terms of microbiological treatment,
- efficient production of reclaimed water, avoiding overdosing of disinfection chemicals and excess of UV power consumption,

- production of fit-for-purpose reclaimed water, according to the intended water quality depending on the water reuse application (e.g., food and non-food crops irrigation, recreational uses, aquifer recharge, industrial applications, etc.).

2 Application

2.1 Matrix

The technology is intended to detect *E. coli* in effluents from WWTP with secondary treatment and with non-chlorinated tertiary treatment (Matrix 1) and River water (Matrix 2).

2.2 Purpose

The purpose of the aquaBio B403 is:

- detect *E. coli* and quantify them expressed as MPN in an automatic and autonomous way in effluent from Wastewater Treatment Plant. The upper limit to be measured is 10^5 MPN/100ml (although the equipment has not an upper limit for calculation).
- detect *E. coli* and quantify them expressed as MPN in an automatic and autonomous way in river water. The upper limit to be measured is 10^4 MPN/100ml (although the equipment has not an upper limit for calculation).
- provide results expressed on a logarithmic scale with a linear regression coefficient higher than 0,95 when compared to the conventional laboratory method ISO 9308-2 (Water quality — Enumeration of *Escherichia coli* and coliform bacteria — Part 2: Most probable number method), and there are no false positive values.
- determine *E. coli* without sample dilution in a minimum time of 5.3 h (for 10^5 MPN/100ml) and a maximum of 12.4 hours (0 bacterium/100 ml) with the same configuration in effluent from Wastewater Treatment Plant.
- determine *E. coli* without sample dilution in a minimum time of 5.3 h (for 10^4 MPN/100ml) and a maximum of 12.4 hours (0 bacterium/100 ml) with the same configuration in river water.

2.3 Conditions of operation and use

Range of application:

- Effluents from WWTP with secondary treatment and with non-chlorinated tertiary treatment,
- River water.

Matrix 1 and 2 sample parameters:

- Turbidity level of matrix: < 200 NTU for all matrices
- Conductivity of matrix: < 5000 µS/cm in all matrices

Reagents used:

- The reagent is a substrate that contains indicator nutrients ONPG (o-nitrophenyl-beta-D-galactopyranoside) and MUG (4-methylumbeilliferyl-beta-D-glucuronide) to detect total coliforms and E. coli to assure the specific growth of the target bacteria.

- Disinfectant solution (a mixture of sulphuric acid (96% solution) and hydrochloric acid (37% solution)

The reagents are stable during the period of one month. In the case of the substrate in a liquid form the possible self-degradation of the substrate is compensated by the system. Just in case of an exponential appearance of the colour or fluorescence is validated by the system.

Limit of determination provided by ADASA SISTEMAS S.A.U.:

- Escherichia coli: 1 bacterium in 100 ml

Analysis time:

- From 5.3 to 12.4 hours (max to get an absence result), depending on the concentration of E. coli

Equipment installation conditions:

- Environmental temperature in between 15 – 30 ºC;
- Sample supply pressure: recommended 0.1 bar; Max. 0.3 Bar
- Vertical suction capacity: max 8 m

Electrical power supply:

- Electrical power supply: 2.47A @ 110 V AC / 1.23 A @ 230 V AC (maximum: 300 W)

Equipment storage conditions:

- 0 – 60 ºC; 5 – 95% RH without condensation

aquaBio B403 setup requirements

- As the kinetics of the microorganisms depends on the stress caused by the matrix, some minor adaptation of the instrument may be necessary for each specific type of water matrix.

2.4 Verification parameters definitions summary

Table 1 summarizes the performance parameters and test methods specified for verification.

Table 1. Specification of the verified parameters

Parameter	**(unit)**	**Test or measurement method(s)**
Performance parameters		
Total culturable heterotrophic bacteria (HTC)	CFU/100 ml	Grab samples collected at the matrices and analyzed at the laboratory according to the pour plate method within 2 h of initiation of the aquaBio B403 sample analysis.
Escherichia coli	MPN/100 ml	Grab samples collected at the matrices and analyzed at the laboratory according to the ISO9308-2 method within 2 h of initiation of the aquaBio B403 sample analysis.
Total coliforms	MPN/100 ml	Grab samples collected at the matrices and analyzed at the laboratory according to the ISO9308-2 method within 2 h of initiation of the aquaBio B403 sample analysis.
Parameter	**(unit)**	**Test or measurement method(s)**
Operational parameters		
Temperature	ºC	Measurement at the matrices with calibrated temperature

		sensor
Turbidity	NTU	Grab samples collected at the matrices and analyzed at the laboratory according to nephelometry method.
Conductivity	μS	Measurement at the matrices with calibrated conductivity meter.
Environmental parameters		
None to be verified		
Additional parameters		
Energy consumption	kWh/d	Calculated based on daily energy demand of aquaBio B403

3 Test and analysis design

3.1 Existing and new data

Based on the technical review of the Verification proposal and the conclusions from the review referring to the assessment of the existing test data provided by ADASA SISTEMAS S.A.U. together with the application, a conclusion was made by the verification body that the verification of the declared performance as specified in the Verification proposal requires generation of new test data.

3.2 Laboratory or field conditions

The samples were collected from 20th of October 2021 to 5th of October 2022. The samples were collected and registered with the location of the sample (effluent of WWTP or river water) and the samples were taken manually. The samples of the following matrixes were collected:

Matrix 1: non-chlorinated treated effluent of a WWTP: Effluent EDAR Gavà, effluent EDAR St. Feliu and effluent EDAR Besós

Matrix 2: river water: River Llobregat and River Besós.

3.3 Matrix compositions

The matrices during the testing were non-chlorinated effluent from wastewater treatment plant and river water.

3.4 Test and analysis parameters

Grab samples of the effluent of WWTP and River water were collected three times a week. The tests were done in the AMB laboratory, located in the wastewater treatment plant of Besòs, operated by Aigües de Barcelona (AB). Once in the laboratory and from the original sample an aliquot was taken in a sterile bottle to be tested for physio-chemical analysis (turbidity and conductivity). If turbidity value was lower than 200 NTU and/or conductivity was lower than 5000µS/cm, the rest of the original sample was split in two different samples collected both in sterile bottles: one was tested for total heterotrophic microorganisms (HTC) and to *E. coli* testing according to the ISO9308-2 method. The other aliquot was used as a sample in the aquaBio B403.

The following numbers of samples were tested during the aquaBio technology testing process:

- Blank samples: 15 samples/15 results

- Matrix 1: 32 samples (aquaBio: 32 results, Colilert: 62 results, including 35 suitable for calculation; rejected 27)
- Matrix 2: 28 samples (aquaBio: 28 results Colilert: 77 results, including 58 suitable for calculation; rejected 21)

3.5 Parameters measured

See the table of results.

4 Verification results (performance, operational and environmental parameters)

Linear regression coefficient analysis

The provided data (MPN) were expressed as logarithm. The numbers obtained in such way were compared (Figure 2 and Figure 3). The assumption of linearity was taken.

$$y=ax+b \qquad [1]$$

Additionally, the b-parameter in formula [1] was fix as zero (no bias).

The linear regression coefficient of provided laboratory data was calculated according to formula:

$$a = \frac{(\sum x_i^2)(\sum y_i) - (\sum x_i)(\sum x_i y_i)}{N(\sum x_i^2) - (\sum x_i)^2}$$

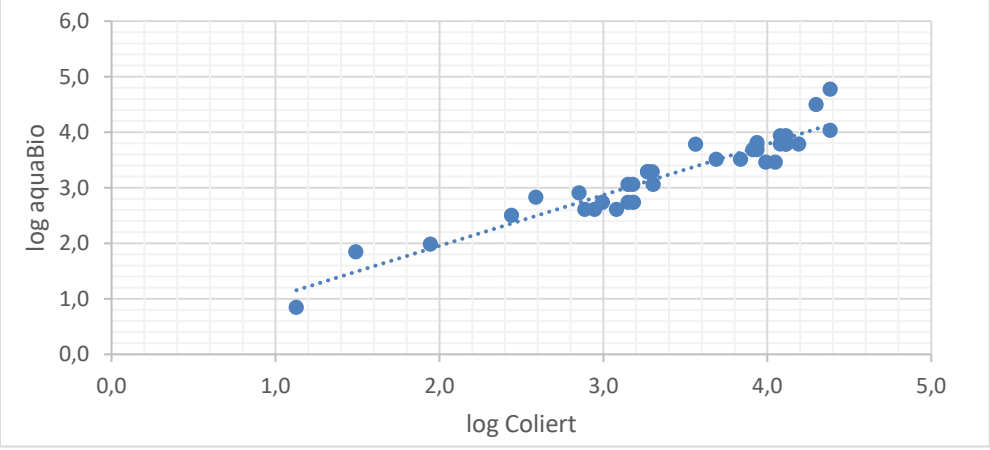

Figure 2. The linear regression for Matrix 1: non-chlorinated treated effluent of a WWTP

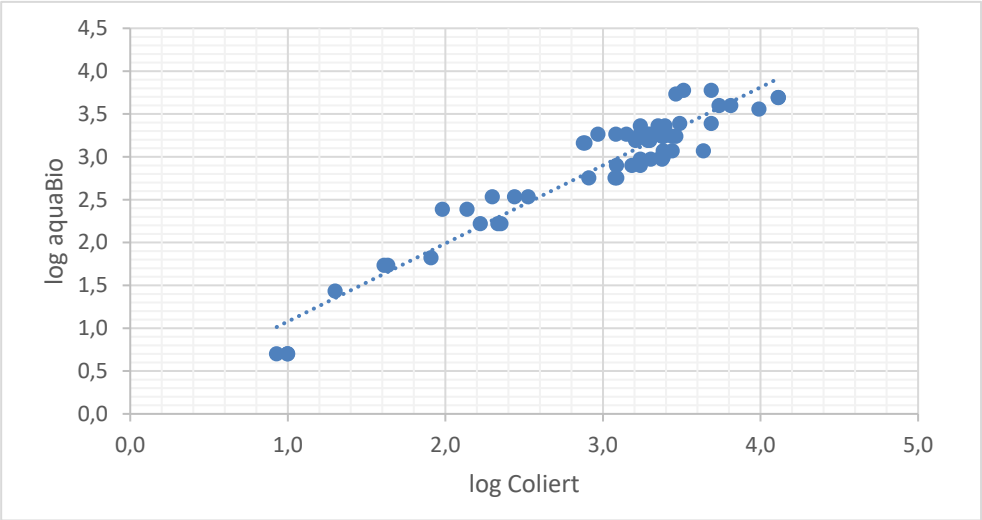

Figure 3. The linear regression for Matrix 2: river water

The linear regression coefficients calculated for both matrices (Figure 2 and Figure 3) are equal:

Matrix 1: a = 0.95

Matrix 2: a = 0.96

and both are above 0.95

No false positive and false negatives values were indicated.

Parameters of tested samples and assumed detection time and conditions of analysis

Table 2 and Table 3 present a summary of the results of the E. coli analysis, taking into account the bacteria detection time and the temperature at which the sample was incubated for methods.

Table 2. Comparison of E. coli detection time for aquaBio B403 and the reference method

		Matrix 1 non-chlorinated treated effluent of a WWTP		**Matrix 2 River water**	
		Reference method	**aquaBio B403**	**Reference method**	**aquaBio B403**
Reaction temperature (for samples ≥ 20 MPN/100mL)	Incubation temperature according to the procedure	**36±2°C**		**36±2°C**	
	Temperature range for the start of incubation	36°C – 36.6°C		36°C – 36.2°C	
	Temperature range for the end of incubation	36°C -36.3°C		36°C -36.2°C	

Incubation time	Incubation time according to the procedure	**18-22h**	**For E. coli 0 MPN/100 mL: 12.4h (744 minutes)**	**18-22h**	**For E. coli 0 MPN/100 mL: 12.4h (744 minutes)**
	Minimum and maximum incubation duration	18h 55min – 19h 45min	744 minutes for sample RG-22/00057 dated 27/06/2022 – for a value of 0 MPN/100mL.	18h 50min – 19h 50min	744 minutes for sample RG-22/00024 dated 28.03.2022 - for a value of 0 MPN/100mL.
			For E. coli 10^5 MPN/100mL: 5.3h (318 minutes)		**For E. coli 10^5 MPN/100mL: 5.3h (318 minutes)**
			320 minutes for sample: RG-21/00003 dated 26.10.21 - for a value of 806615 MPN/100mL.		426 minutes for sample: dated 23.02.2022 - for a value of 10093 MPN/100 mL (Note: Value 10^5 not reached)
Results E. coli (≥ 20 MPN/100mL) Note: the analyzed sample pool was taken for further statistical calculations	The highest number E. coli	RG-21/00007: 24196 MPN/100mL RG-22/00025: 24196 MPN/100mL	RG-21/00007: 59475 MPN/100mL	VA-22/00559 : 12997 MPN/100mL	VA-22/00556: 5943 MPN/100mL
	Lowest number E. coli	RG-22/00003: 30,9 MPN/100mL	RG-22/00003: 70 MPN/100mL	RG-22/00003: 30,9 MPN/100mL	RG-22/00041: 27 MPN/100mL
Results E. coli (< 20 MPN/100mL)	Lowest number E. coli	RG-21/00011: 0 MPN/100mL	RG-21/00009: 0 MPN/100mL	RG-21/00011: 0 MPN/100mL	RG-22/00006: 0 MPN/100mL
		RG-21/00020: 0 MPN/100mL	RG-21/00011: 0 MPN/100mL	RG-21/00020: 0 MPN/100mL	RG-22/00024: 0 MPN/100mL
		RG-21/00009: 1 MPN/100mL	RG-21/00020: 0 MPN/100mL	RG-21/00009: 1 MPN/100mL	RG-22/00034: 0 MPN/100mL
		RG-21/00015: 13,4 MPN/100mL	RG-21/00015: 7 MPN/100mL	RG-21/00015: 13,4 MPN/100mL	RG-22/00027: 5 MPN/100mL

Table 3. The results for blank samples

		Reference method	aquaBio B403
Blank samples *E. coli* (0 MPN/100mL)	Number of samples	15	15
	Results	15 samples 0 MPN/100mL	15 samples 0 MPN/100mL
	Analysis end time	Not applicable	The fastest result of 0 MPN/100mL was obtained after 744 minutes, the latest after 806 minutes

5 Additional information, including additional parameters

The following parameter was declared by the Proposer but was not part of this verification:

Energy consumption:

The energy consumption for the aquaBio measured at 230Vac/50 Hz in a daily mode is 1.32 kW/h and per day. It has been measured with an energy consumption recorder per hour, with an average of 0.05 kW/h, the minimum consumption is 0,04 kW/h and the maximum corresponds to a punctual value of 0.09 kW/h when the sample is introduced in the device.

6 Quality assurance and deviations

The verification was carried out according to the Quality Assurance Plan described in the verification protocol. After the testing, internal and external audits were carried out by TB Àrea Metropolitana de Barcelona (AMB) and Environmental Technologies Verification Body IETU, respectively. There were several deviations to the specific verification protocol and test plan (for details consult the Verification Report). None of the deviations were considered to have significant impact on the verification.
An ex-ante test system assessment performed by the Verification Body has confirmed the reliability of the provided test results.