ETV ISO 14034 ENVIRONMENTAL TECHNOLOGY VERIFICATION

PCA Polskie Centrum Akredytacji INSPEKCJA AK 026

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Statement of Verification

Environmental Technology Verification

Technology Type: **Water Monitoring Technology**

Technology Name: **aquaBio 503**

Statement Registration Number: **JWTŚ-130-3.7/25**

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THE VERIFICATION PROCESS, WHOSE RESULTS ARE SUMMARISED IN THIS STATEMENT, COMPLIES WITH THE ISO STANDARD 14034:2016 ON ENVIRONMENTAL MANAGEMENT: ENVIRONMENTAL TECHNOLOGY VERIFICATION.

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NOTE: . aquaBio 503 is an updated version of aquaBio B403 which performance was verified by ETV Body IETU (Verification Report No JWTŚ-3.5/24) and presented in the Statement of Verification (ETV Body registration number: JWTŚ.130-3.6/24, EU ETV Programme registration number VN20240055 (Statement available under the following address: https://circabc.europa.eu/ui/group/cd5138da-9303-4dec-b82c-bb29409ecdbd/library/485ebc03-6456-4310-9521-65759d3cb90b), date of issuance 15 May 2024. The update consisted in: replacement of the pumps head model from Model WILLIAMSON PUMP 250-420-024/4U-512, peristaltic pump and WILLIAMSON PUMP 250-SMB/6U, Peristaltic pump to pumps with the same technical characteristics: model WATSON; MARLOW, 010.5BU0.00A, peristaltic pump and WATSON MARLOW, 010.5Z2S.00A, peristaltic pump; addition of an extra pinch valve to separate the reagent inlet from the compressed air inlet to facilitate maintenance operation; update of the casing colour from blue to grin and replacement of the ADASA logo with new corporate image of the SKION group; PLC relocation to a new place to optimize space; addition of external sensors to the cuvette to provide alarm in case of sample entrance failure; removal of the power source for safety reasons: aquaBio403 model had a power source to go from 220V to 24V, in aquaBio 503 the power source is external to the equipment. **These changes do not affect the performance verified for aquaBio B403, therefore the conditions applied for verification of aquaBio B403 remain also valid for aquaBio 503. That applies to the use of the Specific Verification Protocol JWTŚ.130-3.3/20, the tests performed and test data reported in the Test Report 902902/23-INF-01 issued by Laboratori de l'Àrea Metropolitana de Barcelona (ENAC accreditation 425/LE854), the Verification Report No JWTŚ.130-3.5/24 as a basis for performance verification of aquaBio 503 and the issuance of this Statement of Verification.**

1 Technology description

aquaBio 503 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 503

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 B503 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 B503 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 503 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 B503 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 503, represent them, consult the proper

functioning of the equipment, and notify the alarms generated in case of exceedance of the configurated thresholds.

aquaBio 503 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 503 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 503 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 503 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.

These parameters and the test methods have been defined for the purpose of performance verification of aquaBio 403 but are also valid for aquaBio 503.

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	°C	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

The test data used for the performance verification of aquaBio 503 is an existing data set generated for the purpose of performance verification of aquaBio B403 following the requirements of ISO 14034:2016. This test data was produced following the Specific Verification Protocol JWTŚ.130-3.3/20 and the related Test Plan, tests were performed and test data was reported in the Test Report 902902/23-INF-01 issued by Laboratori de l'Àrea Metropolitana de Barcelona (ENAC accreditation 425/LE854), the test data was analysed and conclusion on the performance was presented in the Verification Report No JWTŚ.130-3.5/24. The applied test data generation process is presented in sections 3.2 -3.5.

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 B403 technology testing process:

- Blank samples: 15 samples/15 results
- Matrix 1: 32 samples (aquaBio B403: 32 results, Colilert: 62 results, including 35 suitable for calculation; rejected 27)
- Matrix 2: 28 samples (aquaBio B403: 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)

The verification results presented below are these of aquaBio B403. However, since the modifications done to aquaBio 503 do not affect the verified performance, they apply also to aquaBio 503.

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 assumptions 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:

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

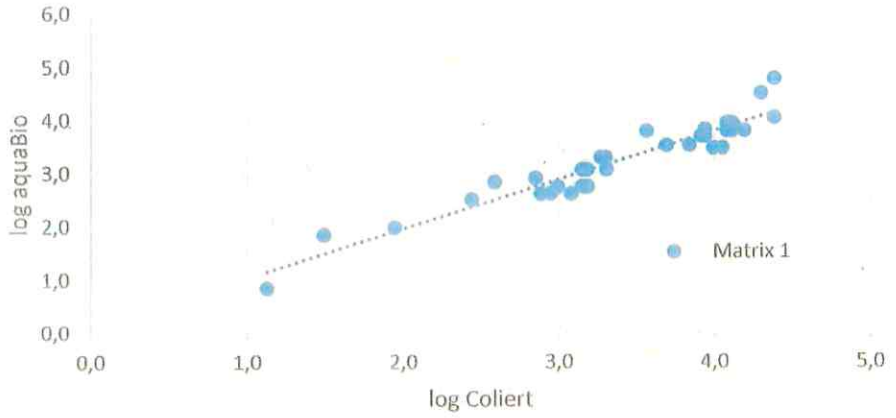

Figure 2. The linear regression for Matrix 1: non-chlorinated treated effluent of a WWTP

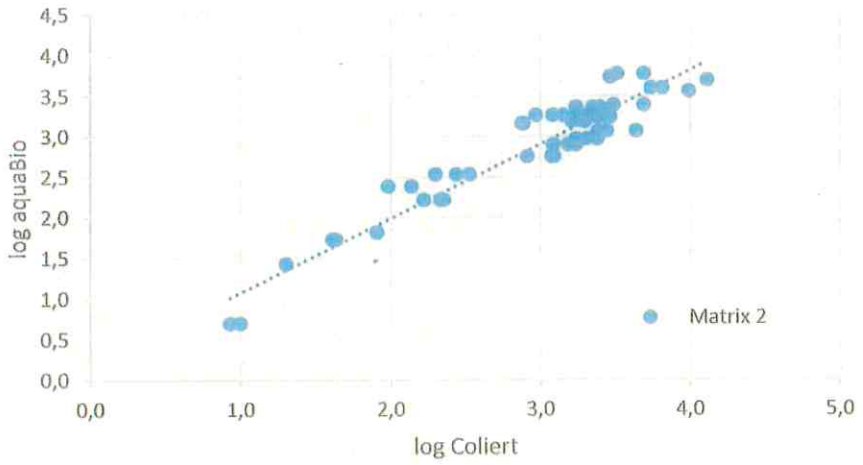

Figure 3. The linear regression for Matrix 2: river water

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[1]

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

Energy consumption:

The energy consumption for the aquaBio 503 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.

Unless stated otherwise, this verification has not evaluated and cannot guarantee compliance with specific legal requirements. Ensuring legal compliance is the responsibility of the Applicant.

[1] with comments or caveats where appropriate