VERIFICATION STATEMENT

In accordance with ISO 14034:2016 – Environmental Technology Verification

ECOLAB | NALCO Water

Technology & Company Information	
Technology Name	**Rapid Bio Intelligence**
Company	**Nalco Water, an Ecolab Company** https://www.ecolab.com/
Technology Type & Application	**On-site assay technology for the detection and enumeration of the total aerobic bacteria population in water samples in cooling water systems**

Verification Parameter	Verified Performance
Aerobic bacterial count comparative results[a]	**Demonstrated -0.52 to 0.10 log_{10} CFU/mL equivalence to the conventional lab plate testing method**
Phone app variability for aerobic bacterial count	**Std dev ± 0.07 log_{10} CFU/mL**
Sample to results turnaround time	**Average 18 minutes**

[a] *For cooling water samples with total aerobic bacterial counts in the range of 10^3 to 10^6 CFU/mL*

Verifier Information	
Verification Body	**350Solutions, Inc.** www.350solutions.com
Lead Verifier	**Bill Chatterton**
Verification Body Accreditation	**ANAB Cert. AI-2618 for ISO:IEC 17020-2012 / ISO 14034-2016**
Verification ID	**VS-EC2001**

*Disclaimer: 350Solutions Name and/or Logo does not imply approval or certification of this product, nor does it make any explicit or implied warrantees or guarantees as to product performance. Information on the performance characteristics of the **Ecolab Rapid Bio Intelligence** can be found at* https://350solutions.com *or at https://www.ecolab.com/offerings/cooling-water-bio-control/rapid-bio-intelligence. Contact 350Solutions or Ecolab to obtain a copy of this ETV verification statement.*

Issue Date:

April 3, 2020

ANAB ACCREDITED ISO/IEC 17020 INSPECTION BODY

350 SOLUTIONS

ENVIRONMENTAL TECHNOLOGY VERIFICATION STATEMENT

Technology Name:	**Rapid Bio Intelligence**
Technology Type:	**On-site Assay Technology for the detection and enumeration of total aerobic bacteria count, https://www.ecolab.com/offerings/cooling-water-bio-control/rapid-bio-intelligence**
Application:	**Cooling Water Systems for Aerobic Bacteria Count**
Company:	**Nalco Water, an Ecolab Company, Naperville, IL https://www.ecolab.com/**
Verification Body:	**350Solutions, Inc. - ISO/IEC 17020:2012 and ISO 14034 Environmental Technology Verification, Certificate Number AI-2619**

VERIFIED PERFORMANCE CLAIMS

Performance data from the Rapid Bio Intelligence on-site assay technology offered by Ecolab was assessed by 350Solutions to verify specific technology performance claims including:

- Comparative results to the conventional laboratory plate testing method for aerobic bacterial count available after 48h incubation,
- Phone app variability for aerobic bacterial count, and
- Sample to results turnaround time.

Verified results for the three technology performance claims are summarized in Table 1.

Table 1 – Rapid Bio Intelligence Verified Performance

Verification Parameters	Verified Performance[a]
Aerobic Bacterial Count Comparative results	Demonstrated -0.52 to 0.10 $\log_{10}$ CFU/mL equivalence to the conventional lab plate testing method
Phone app variability for Aerobic Bacterial Count	Std dev ± 0.07 $\log_{10}$ CFU/mL
Sample to results turnaround time	Average 18 minutes

[a] *For cooling water samples with total aerobic bacterial counts in the range of 10^3 to 10^6 CFU/mL.*

TECHNOLOGY DESCRIPTION

Ecolab's Nalco Water laboratory designed and distributes the Rapid Bio Intelligence On-site Assay Technology for the detection and enumeration of the total aerobic bacteria population in cooling water systems associated with a range of industrial and institutional applications (manufacturing, transportation, food and beverage, paper, chemical, power, metallurgic, and other industries), https://www.ecolab.com/offerings/cooling-water-bio-control/rapid-bio-intelligence. The Rapid Bio Intelligence technology is a mobile app driven test kit designed to provide operators with a means to monitor cooling water quality with a relatively quick and reliable result, allowing operators to implement or alter bacterial control strategies quickly. The test is not strain-specific but provides monitoring of total aerobic bacterial growth in water systems, allowing for quick response to micro-organism contamination. The test is designed to provide an analytical range of 10^3 to 10^6 CFU/ml within a test-to-result turnaround time of 17 to 20 minutes. Test kits include treated test vials, pipettes, and lateral flow strips. Ecolab provides the smart phone application for image capture, processing, and quantitative reporting of bacterial concentrations.

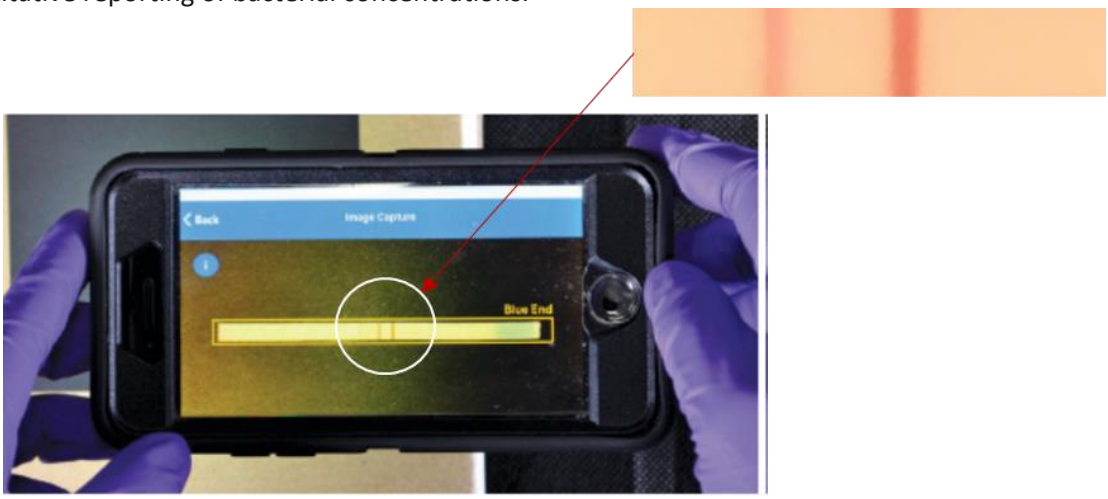


Figure 1. Example image capture using the Rapid Bio phone app.

In use, Rapid Bio Intelligence test strips are read electronically with the Nalco Water E-data mobile app to help ensure consistent and accurate results. The app is compatible with iPhone SE, 6, 6S, 7, 8, X, XS, and newer Apple iPhones. The app is also compatible with Android based phones with HARDWARE_LEVEL_FULL camera2 including Samsung S6 and newer, LG V30 and G6, and other similarly equipped phones. The test kit includes a simple 6-step procedure for collecting and analyzing samples. Users can securely store test results via Nalco's EnVision customer server, can track and visualize historical data, and can identify peak periods of aerobic bacteria levels. The data is readily accessible in user secure EnVision accounts.

VERIFICATION DESCRIPTION

The primary objective of this assessment was to verify the performance claims made by Ecolab with respect to use of the Rapid Bio Intelligence technology in relevant applications. Verification parameters were assessed quantitively using data generated from analysis of cooling water samples with total aerobic bacteria counts in the range of 10^3 to 10^6 colony forming units (CFU)/mL. Verifiers used verified existing technology performance data and additional data generated during the verification process to determine whether the data met the objectives of the verification process. The results of the verification

represent a confirmation of the performance of the technology achieved under the same conditions, constraints and limitations as those specified for the generation of the data used for verification. Ecolab's performance claims specified in the application for verification include:

Performance Claim 1 – Comparative results: For application to cooling water system samples within the effective range of testing from 10^3 to 10^6 CFU/mL for aerobic bacteria, comparative results to the conventional laboratory plate testing method available after 48h incubation are within ± 1-log.

Performance Claim 2 – Phone app variability: Performs consistently (with a relative standard deviation of <5% and a standard deviation of <0.2 for the Log_{10} of the aerobic bacteria counts) using Android phones with HARDWARE_LEVEL_FULL camera2 and iPhone SE, 6, 6S, 7, 8, X, XS, and newer Apple iPhones.

Performance Claim 3 – Sample to result turn-around time: Results provided in less than 20 minutes from start of testing.

After initial reviews of existing test data for the technology and following the Verification Plan, performance verification was specified using two primary sources of information and data: verified existing data generated by Ecolab during technology development, and independent performance data generated during the verification. Using data that was determined to be relevant to the performance claims and of sufficient quality to support verification, 350Solutions assessed the verifiability of the claims. The verification also statistically examined variability and confidence intervals in the supporting data used to verify performance claims including comparative sample analytical results and analytical variability.

For Performance Claim 1, verifiers evaluated comparability by examining the standard error in differences in bacterial count results between the two methods in terms of log_{10} CFU/mL and using the data to conduct a statistical equivalency test. The verification also examined variability within the laboratory method. To assess the Performance Claim of variability with a relative standard deviation of <5% and a standard deviation of <0.2 for the Log_{10} of the aerobic bacteria counts, verifiers examined the results obtained from 15 water samples within the target analytical range of 10^3 – 10^6 CFU/mL analyzed using IOS and Android based phones, along with reference laboratory analyses. Additionally, Rapid Bio reproducibility was assessed by analyzing 6 samples in triplicate using 2 different phones, and reporting the mean, standard deviation, and relative standard deviation. To verify sample to results turn-around time, the time required to obtain analytical results on the phone was reported for each sample analyzed, along with the mean and standard deviation of the sample turnaround times.

VERIFICATION OF PERFORMANCE

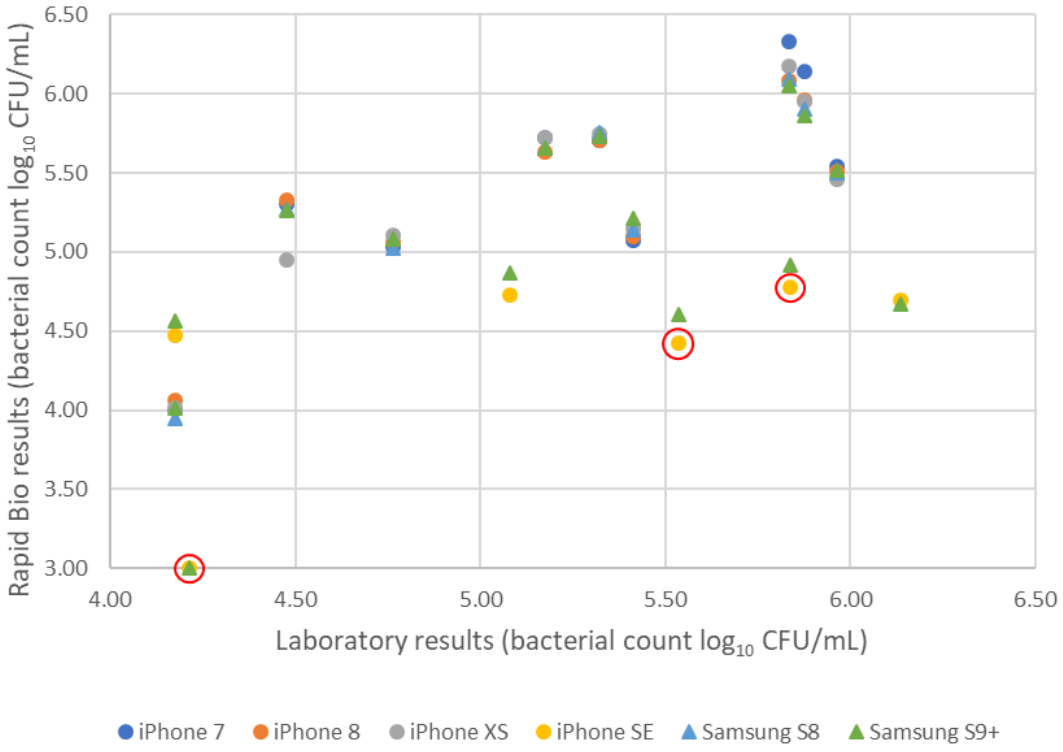
The verification demonstrates that all three of the technology performance claim criteria were met. For comparative results, 15 samples analyzed using a variety of phones and having corresponding verified laboratory results within the range of detection were assessed (Figure 2). Verified results demonstrate that Rapid Bio sample results are in all cases except the four points indicated in Figure 2 within ± 1-log of the laboratory results (that is, 93 percent of results are within the claimed performance specification). Further, results of the statistical equivalence test demonstrate performance well within the equivalency criteria of ± 1-log. Table 2 summarizes the equivalency analysis.

Table 2. Statistical Analysis of Results Equivalence

Method Results				Method Results Difference			
Variable	Mean	StDev	Mean SE	Mean Difference	StDev	Mean SE	95% CI for Equivalence
Rapid Bio Results (log10 CFU/mL)	4.988	0.810	0.209	-0.211	0.688	0.178	-0.524, 0.102
Plate Count Results (log10 CFU/mL)	5.199	0.692	0.179				

The verified standard deviation of responses from 6 different phone types averaged 0.07 CFU/mL log_{10} across the 15 samples used in the analysis and at bacterial count levels ranging from 1.50 x 10^4 to 1.37 x 10^6 CFU/mL. The corresponding relative standard deviation was 1.31%. Both statistical results demonstrate phone app variability well within the performance claims of standard deviation <0.2 for the Log_{10} of the aerobic bacteria counts and <5% relative standard deviation.

Figure 2. Verified Laboratory vs. Rapid Bio Sample Results



The time required to obtain analytical results on the phone apps was verified through direct observations to be in the range of 17.5 to 18.5 minutes over a total of 36 analyses (6 samples analyzed in triplicate using one IOS and one Android based phones). The mean result turnaround time was 18 minutes with a standard deviation of 0.3 minutes.

DATA QUALITY

350Solutions, an ANAB accredited ISO/IEC 17020:2012 inspection body for ISO 14034 ETV, was contracted by Ecolab to provide independent verification of the Rapid Bio Intelligence technology. The verification process applied was based on 350Solutions' Standard Operating Procedure QSP-350-223-01: "ISO 14034 Environmental Technology Verification", the ISO Technical Committee 207 draft guidance document "*Environmental technology verification — E.T.V — Guidance to implement ISO 14034*", and a technology specific Verification Plan. The objectives and approaches used for this verification were designed to apply these principles and processes to Ecolab's application for verification and performance claims. Following ISO 14034 guidance, the data quality assessment included:

- Data quality assessment for the specified performance claims;
- Assessment of ancillary data quality (operations, relevance, representativeness);
- Performer competence (testing and analytical providers);
- Sampling and analytical procedures (repeatability, accuracy, measurement equipment calibration and quality checks); and
- Data management and processing.

Assessment of the quality of data used to verify technology performance was based on three primary components:

- Conformance with the requirements of ISO Standards 14034 and 17025.
- Assessment of the scientific approaches and statistical analyses – specifically, evaluation of measurement uncertainty in laboratory and Rapid Bio test results, the statistical equivalence test used to verify comparative results, and the statistical analyses of measurement variability.
- The quality of reference laboratory procedures and results.

In broad terms, the existing data provided by Ecolab to verify performance with respect to the performance claims were found to be acceptable for verification. The laboratory conducting reference sample analyses was verified as impartial with respect to technology development, and in conformance with the requirements of ISO Standard 17025. Sound scientific approaches and statistical analyses are detailed in Verification Report and demonstrate that the quality of data and data analyses support verification of the performance claims. Assessment of data quality for the reference laboratory results was verified acceptable based on verification of the following data quality assessments:

- Review and verification of acceptable laboratory SOPs and methods,
- Satisfactory verifier observation of laboratory facilities, equipment, and operations,
- Review and observation of performer competence (qualifications and training policies),
- Sampling and analytical procedures (repeatability, accuracy, measurement equipment calibration and quality checks), and
- Satisfactory data management and processing.

All of the findings of the data quality review support verification of the performance claims and conform to the requirements of the standards.

Detailed results of the verification are presented in the Final Report titled *Environmental Technology Verification Report – Rapid Bio Intelligence.* (350Solutions 2020). The report can be made available to the interested parties upon request to Ecolab.

Bill Chatterton

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