Holger Ohme, Dirk Heinecke,
INWATEC

Pascal Jahn, Jennifer Becker
INWATEC

Continuous legionella measurement in cooling water

In-line measuring device for fully automated monitoring of the hygienic water quality in a water sample within a few hours

A newly developed measuring device can measure all legionella species in a water sample within a few hours (Legionella spp. = Species pluralis). For technical water systems from which aerosols can be discharged, the possibility of a reliable rapid test means a decisive advance.

The hygienic need to control the concentration of Legionella in technical water systems from which aerosols can be discharged leads to the problem that the cultivation process (ISO 11731-2017) to be used for this only provides a reliable result with a delay of 7-12 days. On this basis, necessary measures can only be carried out and monitored with a considerable delay. Rapid tests, which are currently available on the market, either do not correlate reliably with the accredited cultivation method or require (time-)consuming processing steps. Some rapid tests provide highly specific evidence for individual Legionella species, but not for all

Legionella species in a water sample (Legionella spp. = Species pluralis). The method of measuring the metabolic activity of living cells, on which the newly developed INWATROL L.nella + measuring device is based, determines the Legionella spp parameter. reliably within a few hours from a water sample. The measuring device is connected directly to the technical water system with automatic and self-disinfecting sample collection, including self-disinfection of the water contained in the measuring cell after the measurement has been completed. This enables the system operator to continuously and safely determine the hygienic water quality. In addition to the direct success control of implemented measures, the needs-based control of e.g. biocides is also possible.

1. Introduction

The hygienic relevance of the spread of pathogenic legionella via aerosols from technical water systems such as evaporative cooling systems and cooling towers has led to the creation of technical hygiene guidelines in many countries. In Germany, VDI 2047 sheets 2 and 3 came into force for the first time in 2015, the generally recognized rules of technology for ensuring the hygienic operation of evaporative cooling systems and cooling towers. In addition, in many countries the tolerable concentration of Legionella in the circuit water of the systems concerned is limited by the legislature. In Germany, on August 19, 2017, the forty-second ordinance for the implementation of the Federal Immission Control Act (ordinance on evaporative cooling systems, cooling towers and wet separators - 42nd BImSchV) came into force, which also includes wet separators.

So far, the basis for the hygiene control has always been the determination of the concentration of Legionella in the water through cultivation in accordance with ISO 11731: 2017 with threshold values defined depending on the system. With the cultivation method, visible and therefore countable colonies arise through cell division. Compared to other types of bacteria, Legionella divide relatively slowly, so that the results of the measurement are only available after 7-12 days, with further examinations sometimes following to confirm suspicious colonies.

For the operator of a system that is subject to mandatory monitoring, this means a greatly delayed control of the hygiene status. The effectiveness of any necessary measures can also only be determined with a long delay. Additional rapid tests for estimating the contamination of the water with Legionella are available based on immunological reactions (antibodies), detection of genetic material (PCR) or using color fluorescence microscopy. The limits of these rapid tests lie in the live / dead quantification, the comparability with the culture method or in the complex sample preparation.

With the newly developed and patented INWATROL L.nella + automatic measuring device, the reliable and continuous determination of the parameter Legionella spp. with a high correlation to the cultivation method according to ISO 11731: 2017 possible within a few hours without further processing steps by the user.

2. Rapid test for the fully automated determination of Legionella spp.

2.1 Measuring principle

The detection of metabolically active Legionella is carried out by a non-specific enzymatic conversion of a non-polar fluorescein acid

Abb. 1: INWATROL L.nella + as wall mounting

ester, which reaches the cell interior exclusively via the cell membrane of living cells and is converted into color-active fluorescein.

The increase in fluorescence as a function of time is directly proportional to the number of living cells and is converted into colony-forming units per 100 ml. The accompanying flora is killed by a combined heat and pH pretreatment as well as the high measuring temperature compared to the cultivation method. The measurement is carried out undiluted in a sample volume of approx. 350 ml. Compared to the cultivation method, the measurement is not significantly influenced by accompanying flora and a high measurement inaccuracy due to high dilution.

The measuring device is connected directly to a water system for continuous measurements. A thermally self-disinfecting sampling line ensures that no legionella proliferation in the supply line affects the measurement result. Ideally, the sampling tap is in continuous operation in order to be able to rule out stagnation of water between two measurements. The measuring cell in the device is rinsed several times during filling. When the rinsing process is complete, the combined heat and pH pretreatment begins.

In addition, the automated dosing of the inactivating agent takes place when a biocide is used. When the pretreatment is completed, the measuring cell cools down to the measuring temperature and the measurement begins. Before the device is refilled for the follow-up examination, the measuring cell is thermally disinfected. The measuring cell is ready for the next measurement. As a rule,

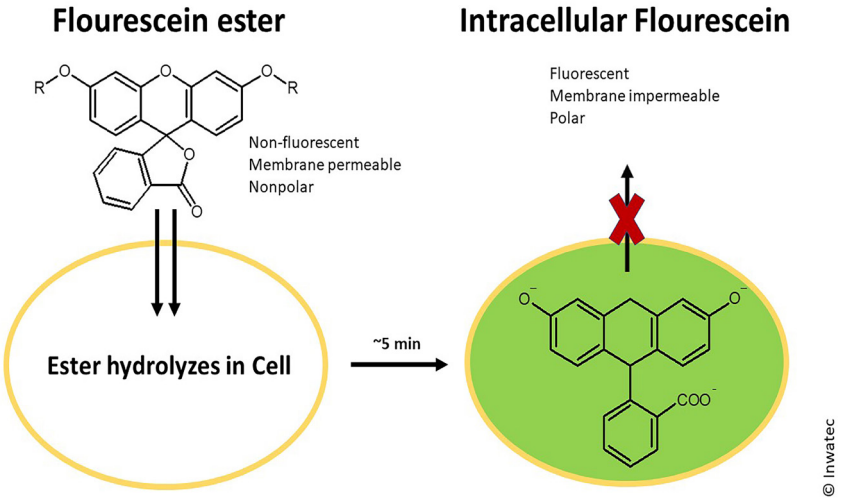

Abb. 2: Measuring principle INWATROL L.nella +

a sampling tap is installed directly at the sampling point in front of the sampling line, which can be used to take microbiological samples, e.g. for further validation measurements, at the time of filling the measuring cell or at any other time.

2.2 Automated measurement of manually applied samples

The continuous measuring operation can be interrupted for the manual addition of further water samples via the filling funnel. To clean, rinse and fill the measuring cell, only the valve position on the device has to be changed. When the filling is completed, the valve position is returned to its initial state and the measuring device switches back to automatic mode when the measurement is completed. The measuring process itself does not differ from automatic mode.

2.3 Cultivation according to ISO 11731: 2017 / UBA

The cultivation method uses several approaches with different levels of dilution and pretreatment (heat or acid). The aim is to obtain an evaluable result for both low and high levels of Legionella.

The batch with the highest number of confirmed Legionella colonies is used for the result (if the measurement accuracy / number of colonies is sufficiently high). The limits of the accuracy of the cultivation method lie primarily in the possible influence of accompanying flora, i.e. other microorganisms that suppress the growth of Legionella or can overgrow their colonies.

In addition, bacteria are particles in a water sample and are not distributed homogeneously. When small volumes are withdrawn from the sample bottle, inaccuracies can occur due to the sometimes high dilution factors.

During cultivation, living but non-cultivatable cells in the so-called VBNC status are not recorded. Many legionella from a coherent agglomerate, e.g. through reproduction within an amoeba, are only visible and assessed as a colony during cultivation (see Lindner, Hahn: Microbiological analyzes of the cooling water according to the 42nd BImSchV, p. 74, VGB PowerTech 9, 2018).

3.3 Correlation of the INWATROL L.nella + rapid test with the cultivation method

The correlation of the rapid test was carried out over a large number of measurements with the cultivation sample in accordance with ISO 11731: 2017. The implementation of sampling, sample transport as well as preparation and evaluation of the measurement results were carried out in accordance with the current recommendation of the Federal Environment Agency for sampling and for the detection of legionella in evaporative cooling systems, cooling towers and wet separators (UBA).

Validation measurements were carried out with different accredited laboratories. In order to obtain a reliable qualitative comparison between the rapid test and the cultivation method, the measurements shown below were carried out in only one accredited test laboratory (IWW Rheinisch-Westfälisches Institut für Wasser Beratungs- und Entwicklungsgesellschaft mbH, D-45476 Mülheim an der Ruhr).

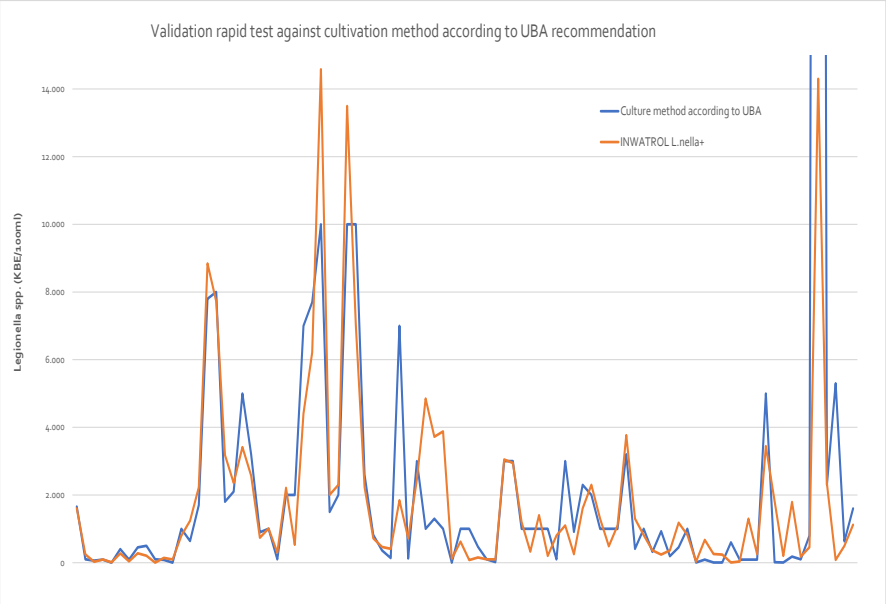

Abb. 6: Validierung Schnelltest gegen Kultivierungsmethode

4. Discussion

The correlation to the cultural approaches carried out in the laboratory can be rated as very high overall. Two devices briefly showed significant deviations from the laboratory results in the form of additional findings. Here the influence of VBNC cells on the measurement result of the INWATROL L.nella + rapid test was examined. Metabolic activity measurements using fluorescein diacetate are used in microbiology in addition to other methods (membrane integrity, protein synthesis (FISH), „intact polar membrane lipid" analysis, cell elongation („direct viable count")) for the detection of VBNC bacteria.

This can represent an additional benefit for the operator, since recontamination of water systems with Legionella can also be a „revival" of VBNC organisms (cf. Hans-Curt Flemming, Jost Wingender - IWW Center Water, Biofilm Center, University of Duisburg -Eat). Often, however, the operator's goal is to achieve the highest possible correlation with the legally mandatory examination by means of cultivation in the laboratory.

To be achieved through the legally compulsory examination by cultivation in the laboratory. By adapting the pretreatment conditions (especially by increasing the temperature and extending the pretreatment time), the correlation to the cultivation method can be successfully restored in the event of multiple findings from VBNC cells.

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Biofilme im Trinkwasser – Schwachpunkt Hausinstallation, Hans-Curt Flemming, Jost Wingender -IWW Zentrum Wasser, Biofilm Centre, Universität Duisburg-Essen

Phone:
+49 2271 99551 0
E-Mail:
info@inwatec.com
legionellen@inwatec.com

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