New methods for on-line detection of coliforme bacteria in drinking water

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Abstract. During our work at the water supplies, we have obtained practical experiences with a number of fast detection methods for microbial quality. In this work we focus on fast methods for detection of coliforme bacteria and to a lesser extend Escherichia coli (E-coli). Our experiences include the following methods: The process analyzers mBonline Coliguard® and Colifast ALARM® and the operational tools "Integrated Sample Unit" where the laboratory method Colilert-18®/Quanti-Tray® is used to quantify the coliforme and E-coli bacteria.

The methods will be described with regards to detection limits, response time, operational qualities, and limitations to operation. The process analyser Colifast ALARM® has been on the market for a couple of years and is used as a part of daily operation control at Københavns Energi, whereas Coliguard® is new on the Danish market. The three water supply companies of Copenhagen, Roskilde, and Gentofte/Gladsaxe, and the company Amphi-Bac have in collaboration tested the analyzer directly in our full-scale production facilities with regards to detection limits, response time, operational qualities, and limitations to operation.

Introduction

In Denmark, groundwater covers almost 100% of our need for drinking water. It is a political decision that the drinking water should be treated as simple as possible before it reaches the taps of the consumers. Typically, treatment consists of aeration and filtration (removal of iron and manganese) at the water works before the water is pumped into the distribution net to the consumers.

A barrier such as chlorination of drinking water is not common practice. Thus, in order to insure healthy drinking water with good bacterial and physical/chemical quality it is critical to have fast methods, which can monitor drinking water quality during production and distribution. Water works, researchers, and consultants are constantly challenging the limits for detection and delivery of results developing new methods for rapid microbial quality control.

During our work at the water supplies of Copenhagen, Gentofte/Gladsaxe, and Roskilde areas, we have obtained practical experiences with a number of fast detection methods for microbial quality. The purpose of this presentation is to share our experiences with on-line methods installed directly at outlets from our waterworks. The focus is on coliforme bacteria and to a lesser extent Escherichia coli (E-coli). Our experiences include the following process analyzers: Coliguard® and Colifast®, and the operational tools "Integrated Sample Unit", where the laboratory method Colilert 18®/Quanti-Tray® is used to quantify the coliforme and E-coli bacteria. All techniques have the potential to establish an important part of continuous operation and control of microbial quality in drinking water production.

These methods are developed to monitor the sanitary quality of water upon detection of indicator bacteria such as coliforme bacteria and E-coli as fast as possible. As mentioned the Danish water works are privileged with a relatively good microbial raw water quality. Without barriers as chlorination etc. water of good sanitary quality is supplied. In normal situations the detected level of coliforme bacteria is low from the water works to the consumer. Higher levels are used in the operation and control procedures as a warning signal for leakage, accidental pollutions, failure in treatment processes etc. In these situations we are challenged in time and the need for faster methods is obvious.

Methods

"Integrated Sample Unit"

In the Danish water supply sector the standard methods ISO EN 9308-1:2001 and Colilert®/Quanti-Tray® are used for detection of coliforms and E-coli. Grab sampling with a sample size of 100 ml. is the common procedure. The use of large sample volume and longer sampling periods improve the sensitivity in the detection of coliforms in our systems.

A sampling unit with the possibility to take out larger samples and use longer sampling periods has been integrated in the operation and control at the waterworks cooperating in this project. This sampling unit is named "Integrated Sample Unit"

Figure 1: "Integrated Sample Unit" **Figure 2:** Extraction of coliforme bacteria from filter paper

In the sampling unit the sample is filtered through a sterile filter unit. The coliforms are caught on the filter surface. After a determined filtration time and -volume the filter-unit is removed and transported to the laboratory. The coliforms are extracted from the filter and the number of coliforms is analyzed using the Colilert-18®/Quanti-Tray® method. By filtration of 100 litres of water sample during 24 hours the detection limit of coliforms and E-coli is improved 1000 times compared to normal procedure with grab sampling of 100 ml. This sampling unit was installed at the test points in order to compare the results of the Coliguard® and the Colifast® with the Colilert® method.

Colilert-18®/Quanti-Tray®

The enzyme substrate test from US Company IDEXX was used for simultaneous detection of coliforms and E-coli numbers in the spiked water samples in our tests. Also in connection with the use of the sampling unit in the operational tests of the process analyzer.

Figure 3: Quanti Tray® wells from the Colilert® MPN method

Colifast process analyzer ALARM®

The Colifast ALARM® is developed and produced by the Norwegian Company Colifast AB. The company has been working with automation of methods for bacteria detection and measurement in food and water for the past 20 years.

The Colifast ALARM® is a stand-alone at-line early warning system developed for detecting either one of the three following microbial parameters: Total coliforms, faecal coliforms, or E-coli in 100 ml water samples.

Detection of target bacteria is based on selective growth in Colifast® medium and the hydrolysis of fluorogenic substrates by specific bacterial enzymes, which ensure accurate results, detecting only cultivatable bacteria of the target group.

The analyzing time for the sample is 15 hours. After this time a final result for the sample is reported. If the result is positive coliforme bacteria is present at a level above 1 coliform/100 ml, and below 1 coliforme/ 100 ml if the result is negative. The first measurement on the incubated sample is performed after 6 hours (early warning for high levels of coliforms) and the system continues to measure every hour until the final 15 hours.

The result is then reported by following options decided by the end user: SMS, LAN and PLC interfacing and audio-visual alarms at the local position of the actual analyzer-system. Københavns Energi decided to buy the system after a trial project in 2010.

It has been operating continuously with good and stable results since June 2010, at a control point at the water storage facility for Copenhagen, Tinghøj. It runs every day all year around. A major advantage developed is the possibility to do testing at night, where no manual sampling and following laboratory analysis can be provided under normal conditions. By the use of the system, incidents with abnormal high level of coliforms have been detected with following trouble shooting in our systems to find the reason.

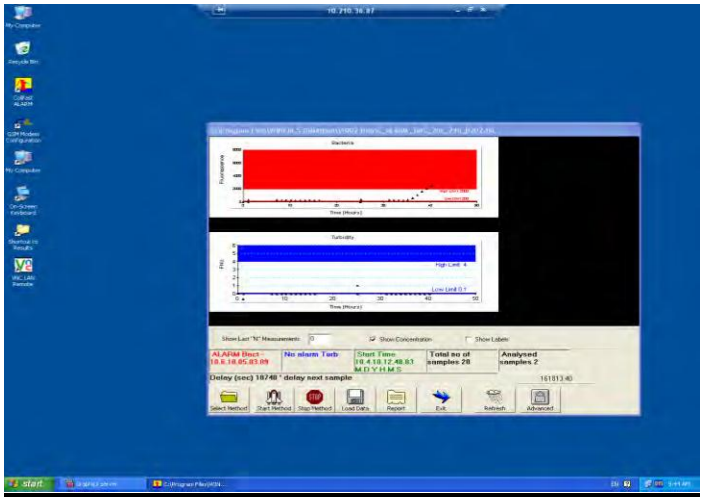

Figure 4 and 5: The Colifast ALARM installation at Københavns Energi, Tinghøj water storage facility. "Remote Desktop" operation. Example of an unusual situation. Detection of high level of coliforms

mBonline Coliguard® process analyzer

The three water supply companies of Copenhagen, Gentofte/Gladsaxe, and Roskilde, have in collaboration tested Coliguard® from the Austrian company MB-Online GmbH founded in 2008, which is a new on-line method for monitoring coliforme and E-coli bacteria. The Coliguard® method can deliver a result in three hours, which to the writers' knowledge is the lowest response time to date. Coliguard is already used at drinking water supplies in a number of countries in Europe, however; so far it is mostly used on drinking water resources originating from surface water, which contains much higher concentrations of bacteria than ground water. Thus, in order for Danish water works to be interested in this new method a low detection limit is a key issue. As a part of the test of MB-online Coliguard®, results have been compared with traditional microbial methods, which were used in parallel for comparison.

In order to make the study more applicable to everyday operation at the water works, the apparatus was tested directly in our full-scale production and distribution systems. So far, it has not been possible to find automated or manual methods, which can deliver a reliable result in such a short time span. We were therefore eager to see whether this tool was able to fulfil the promised specifications and our expectations.

The full automatic system can either detect low levels of coliforms or E-coli by measuring their biochemical metabolic rate during fluorescencoptical measurements.

Figure 6: The internal operation side of the mB-Online Coliguard® analyzer

The enzymatic activity of coliforms is detected by measuring the concentration of β-galactosidase, whereas E. coli are determined by measuring the concentration of β-glucuronidase, an enzyme, which is a selective marker for E-coli. Results are typically delivered directly to our SCADA-systems in three hours, which is the major advantage compared to the standard control methods.

The sample volume can vary in a range up to 3000 ml; in our test it was preset to 2000 ml. The sample is filtered through a ceramic filter, where after the filter sample is mixed with either coliforme- or E-coli-reagent. After each measurement the ceramic filter and reaction chamber is cleaned with a cleaning solution.

The mboClient is a JAVA based application that can be installed on any PC. The user face is user friendly, besides the presentation of the measuring results in a clearly arranged diagram, instrument parameters can be displayed and adjusted. The module „Timetable "allows the setup of a sophisticated schedule when measurements are to be performed, the system status informs about the remaining quantities of consumables. An integrated export module permits the export of data e.g. into an excel spreadsheet for further processing.

The Coliguard apparatus was installed and ran continually for six months at Critical Control Points at two water works in Hørsholm and Roskilde, and at the Tinghøj storage facility.

Figure 7: Operational test of the Coliguard analyzer

The background enzyme level of β-galactosidase was stable at each location, but varied with locations. At Tinghøj the level of β-galactosidase correspond to 1,4 coliforms/100 ml with the conversion factor being used, but no β-glucuronidase background was detected. At Haraldsborg and Sjælsø the levels of β-

galactosidase correspond to 1,6/ 100 ml and 2,0/100 ml neither at these two sites any β-glucuronidase background was measured.

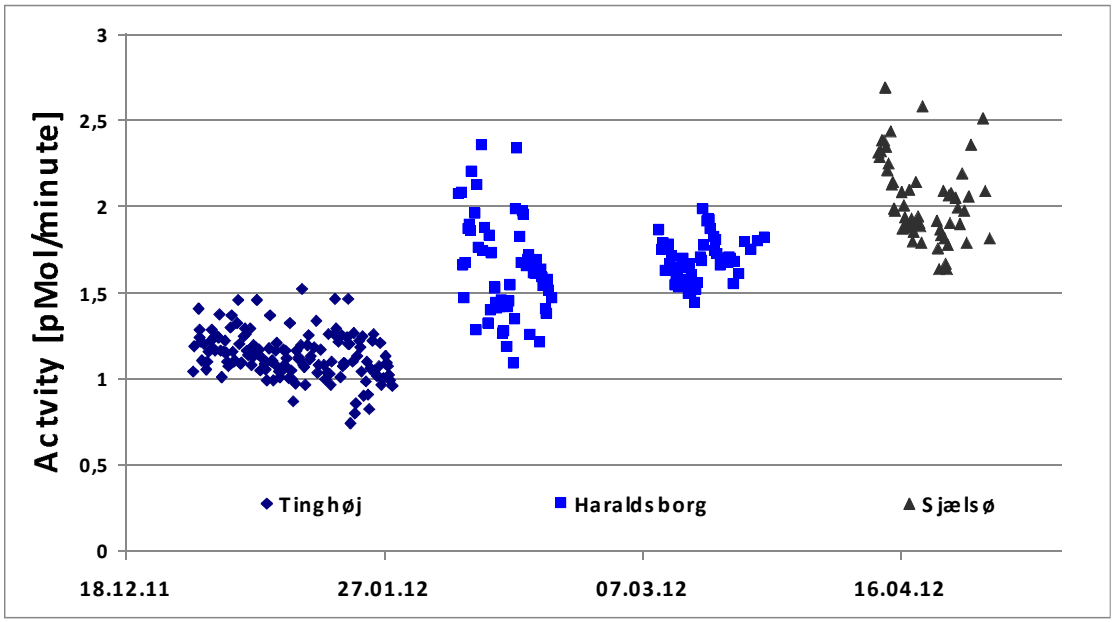

Figure 8: Background enzyme activity levels at the three water facilities during the test period. Simultaneously Colilert analysis didn't show presence of coliforme bacteria or E. coli.

The water was simultaneously been analyzed using the "Integrated Sample Unit" followed by a Colilert® measurement on the filter wash off. The Colilert analysis did not show the presence of either coliforms or E-coli at any of the three sites. The reason for the variation of background β-galactosidase level may be due to the presence of other microorganisms such as protozoa and other bacteria.

Spiking experiments

Because no true water contamination occurred through the testing period, three types of spike tests were carried out. The first spike test was carried out at Tinghøj. This was done by spiking 20 L of drinking water from the Tinghøj storage facility, with freeze dried coliforme bacteria with a certified number of bacteria. In order to verify the concentration two water samples were simultaneously analysed by the Colilert method. The results of the Colilert method were 6 coliforms/ 100 ml.The first spiking experiment showed clearly a difference between background level and the 'contaminated' level, which was very promising.

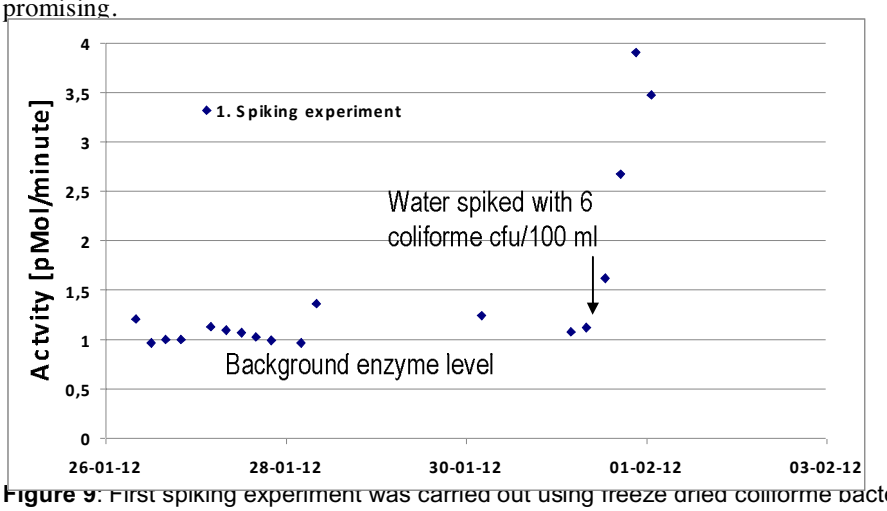

Figure 9: First spiking experiment was carried out using freeze dried coliforme bacteria. Colilert analysis showed a concentration of coliforme bacteria at 6 cfu/ 100 ml.

In the second spike test, we were interested in testing the detection level of the Coliguard apparatus. In this test water from the Tinghøj sub container number 6 was analysed. Sub container number 6, which is closed down for repair, contained water contaminated with coliforme bacteria at concentrations of 1-2 coliforms/ 100 ml. 20 L was twice analysed by the Coliguard® apparatus. At the beginning of each test, water samples were analysed by the Colilert method.

The result of the first analysis (2.1) of Tinghøj-water from sub container number 6 showed an increase in enzyme level to a concentration 2 times above background level. At the second analysis (2.2) the enzyme level didn't increase above background level. We cannot explain the reason for this difference. In order to assure that it isn't a malfunction of the apparatus the Coliguard instrument is currently in for testing.

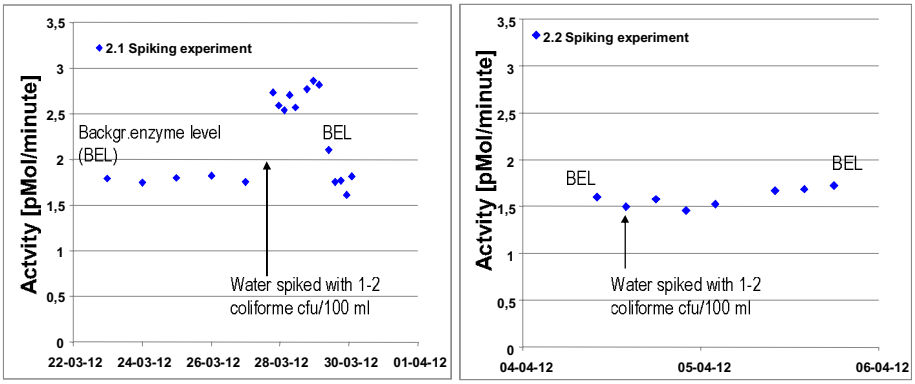

Figure 10 Second spiking experiment, which consists of two repetitions. Simultaneously Colilert analysis showed the presence of 1-2 coliforme bacteria. BEL: background enzyme level

In the third experiment we wanted to examine whether there was a linear dependence in enzyme activity and coliforme as well as E. coli concentration, furthermore, we wanted to test the detection limits once again. An spike sample of 20 litres with "a target concentration" of around 20-30 coliformes/100 ml was made by adding fresh wastewater to drinking water taken from the tap. (Dilution factor around 1/40000).This sample was analyzed for typical water parameters and microbial quality parameters.

During a period of three days a 20 l solution of strongly dissolved wastewater (1/40000) was stepwise added drinking water resulting in lower and lower concentrations of coliforms and E-coli. The solution was monitored during the three days, each day the Coliguard® apparatus performed two analyses for coliforme bacteria followed by two analyses for E-coli. At the end of each day a known volume of drinking water was added to the left over volume of solution resulting in a dissolution series of approximately 1, 5/6, and 1/4. Thereby, approaching the detection limits of the Coliguard® apparatus. This approach would also allow us to examine whether the enzyme activity of the bacteria would follow the concentration levels detected by the Colilert method.

The sample of waste water dissolved into 20 L of drinking water resulted in an average concentration of coliforms at 25/ 100 ml and E-coli at 7/ 100 ml according to the corresponding Colilert analysis. Due to the-stability of the bacteria it was necessary to decrease the addition of drinking water on day 2 and 3 to the absolute minimum in order to ensure enough bacteria to measure. In each sample- round two samples were taken both of which were analysed twice. The average result of each sample is shown in the table below.

Table 1 Third spiking experiment was carried out over a period of three days. Simultaneously Colilert analysis showed the presence of coliforme bacteria and E. coli.

	Colilert (coliforms/100 ml) Double sampling	Colilert (E-coli/100 ml) Double sampling	Coliguard (coliforms/100 ml) *	Volume in container (litres)
Background	<10	<1	1,9	
Day 1 start	34/ 16	8/ 6	2,1	20
Day 1 end	15/ 15	6/3	2,1	10
Day 2 start	12/ 10	3/ 4	2,1	12
Day 2 end	13 / 13	4/ 5	2,2	2
Day 3 start	8/ 10	1/ 4	2,2	12
Day 3 end	27/ 15	1/ 2	2,1	2
Background	< 1	<1	2,4	

* Coliguard result. Calculated from enzyme activity and conversion-factor for coliforme

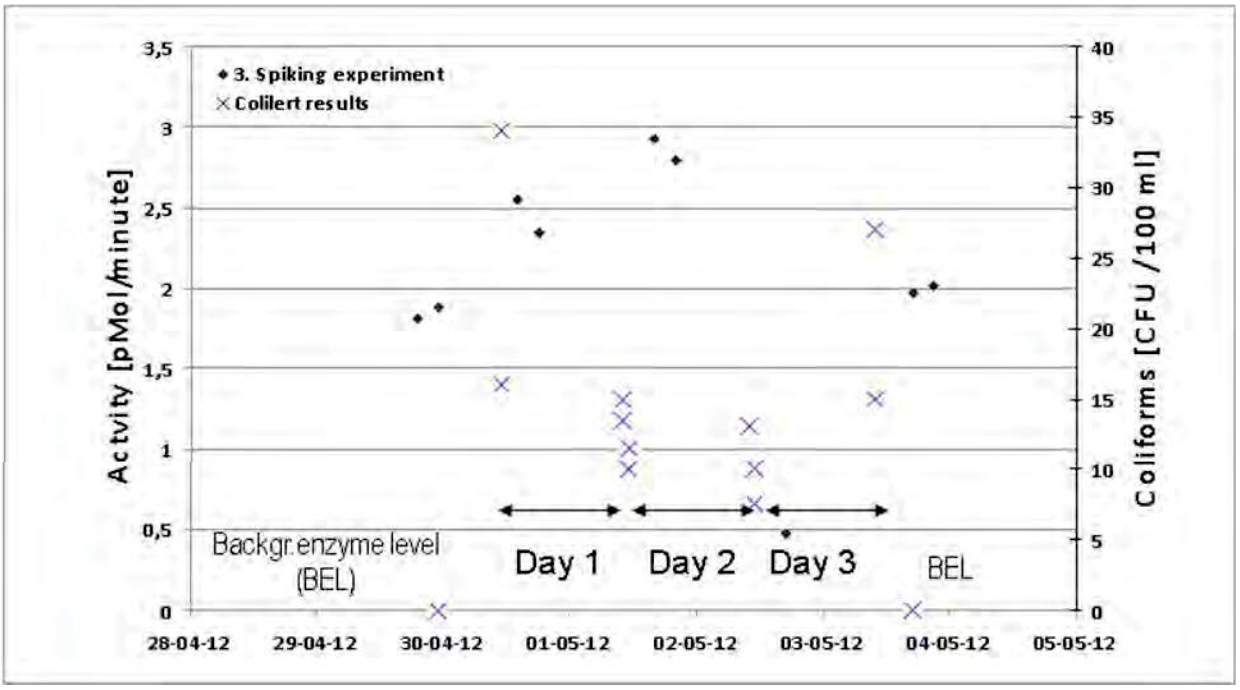

Figure 11: ***Coliforms***. Third spiking experiment was carried out over a period of three days. Simultaneously Colilert analysis showed the presence of coliforme bacteria according to the table above. BEL: background enzyme level

According to the Colilert method the bacterial community changed a lot during the three days of the experiment. During the first day the concentration of coliforme and the E. coli bacteria decreased. On the second day the numbers of coliforme and E. coli increased during the day. On the third day coliforme increased, but E. coli decreased. This instability of a bacterial wastewater community is not surprising; however, it was not possible to recognise the pattern in the results of the Coliguard.

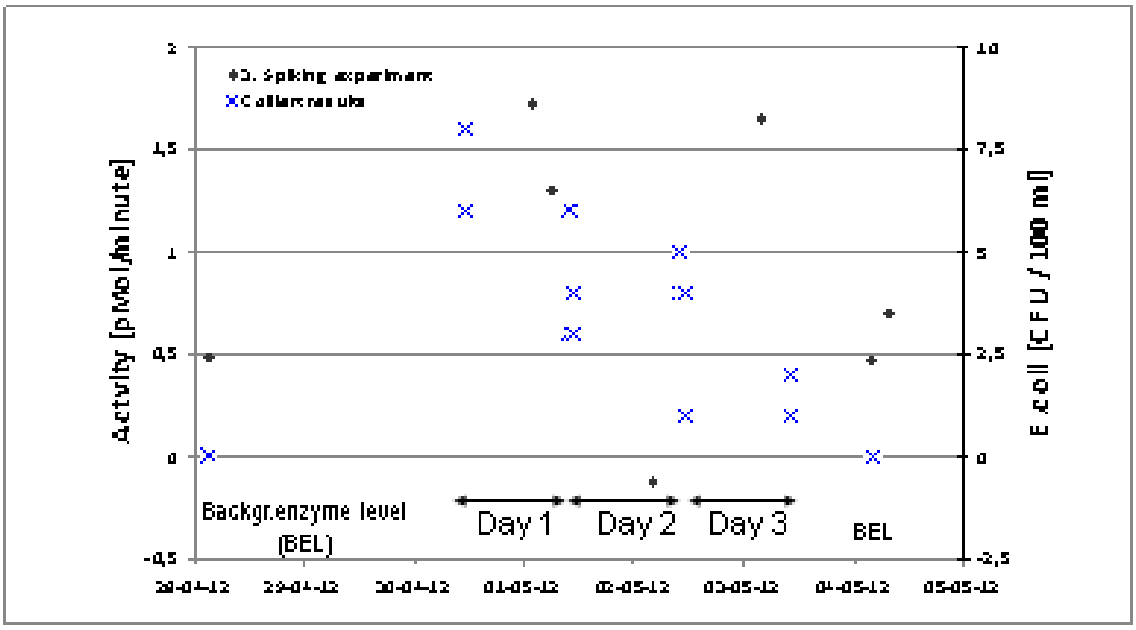

Figure 12: ***E-Coli***. Third spiking experiment was carried out over a period of three days. Simultaneously Colilert analysis showed the presence of coliforme bacteria according to the table above. BEL: background enzyme level

It was not possible in the third experiment to distinguish between the background level and a low level contamination with up to 34 coliforms/ 100 ml and 8 E-coli/ 100 ml. This result is puzzling because it seemed possible to distinguish levels at 6 and 1-2 coliforme bacteria in the first two spiking experiments. Furthermore, AmphiBac reports to have measured levels of 1-10 coliforme bacteria in own tests.

The Coliguard apparatus has been sent to a thorough testing in order to check it for malfunctions.

However, if the Coliguard isn't capable of distinguishing between low levels of coliforme bacteria it is very unfortunate in a Danish context, because legal limits are low.

Conclusion

Several incidents of contamination of drinking water with Coliforme bacteria makes it clear that there is a need for rapid methods for detection of unwanted bacteria in our drinking water.With regards to coliforme and E. coli bacteria we have obtained practical experiences with three on-line methods: Colilert 18, Colifast, and Coliguard.

Colilert 18 and Colifast are based on selective growth whereas Coliguard is based on measurement of enzyme activity. Selective growth techniques require a much longer time span for result delivery. In both cases it takes 18 hours to obtain a result, however, Colifast provides the option for an early warning after 6 hours in case of a major contamination. Because Coliguard is based on measuring the enzyme activity it is possible to deliver a result in three hours. On the downside enzyme activity based techniques aren't as precise as selective growth techniques, which again only focus on a fraction of bacteria that is growth able.

Colilert 18 and Colifast are used in the daily operation, and deliver steady and reliable results, and are easy to operate and maintain. Coliguard has a more advanced user interface than Colilert 18 and Colifast, which provide the user with several options for set-up.

The test for whether Coliguard is able to reliable results in the low contamination level range is not conclusive. The first half of the test showed positive results whereas the last half showed negative results. In order to rule out instrument malfunctions the instrument is in for a thorough inspection which will be done before the conference presentation.

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