New Paths in the Analysis of Drinking Water Quality

The Tedious Search for a Fast Alternative Method

Drinking water is routinely tested for the presence of bacteria by culturing methods which have the disadvantage of being very time consuming. It takes at least one day before results are available. For this reason, EAWAG is currently developing a faster method. The application of new molecular techniques is promising, although the development of an actual method is proving to be rather difficult.

The hygienic quality of drinking water has been routinely tested for many decades now by determining several bacterial parameters. These tests typically screen for so-called indicator organisms, such as the intestinal bacterium *Escherichia coli* [1]. The assumption is that these harmless bacteria are secreted along with potential pathogens and could be introduced into drinking water supplies. A simple and inexpensive culturing method for the detection of *E. coli* has been used for many decades. However, this method is rather time consuming: it takes at least 24 hours before results are available. There are situations in which the water supplier would like to be able to determine more rapidly whether or not the water delivered to customers is hygienically acceptable. It is possible, for example, that after several days of intense precipitation, one or more of the sources of drinking water receives poorly filtered water. In a worst-case scenario, this could lead to fecal matter-contaminated drinking water. Were this the case, the drinking water supplier would have to immediately take measures to decontaminate the drinking water supply (disinfection, flushing) and would also have to advise the population to boil water before use. When it takes 24 hours before test results are available, it may be too late for the appropriate response. For this reason, EAWAG is developing a faster detection method that is based on newer molecular methods of analysis.

The *E. coli* Reference Method

Drinking water is considered a “food” and is, therefore, subject to the Swiss Ordinance on Hygiene [2]. The ordinance specifies that drinking water quality must be assessed using the reference methods defined in the Swiss Food Manual [3]. The use of other methods is permissible if “they were proven to lead to the same interpretation as the reference method” [2]. The reference method for the detection of *E. coli* in drinking water is the culturing method (Fig. 1, top). The Ordinance on Hygiene sets the tolerance for *E. coli* at “not detectable in 100 ml water sample”. Therefore for the culturing method, 100 ml of water are passed through a filter, on which *E. coli* and other organisms are retained. The filter is then laid on an agar plate where individual bacterial cells grow to colonies that can be enumerated. Whether a particular colony is, in fact, a colony of *E. coli*, is confirmed by an enzymatic reaction whereby *E. coli* colonies turn blue in color.

The Newly Developed Alternative Method

In the search for faster alternative methods, techniques from molecular biology have received much interest. In our opinion, the use of the “Polymerase Chain Reaction” (PCR) is particularly promising (Fig. 1, bottom). In this method, short DNA segments charac-

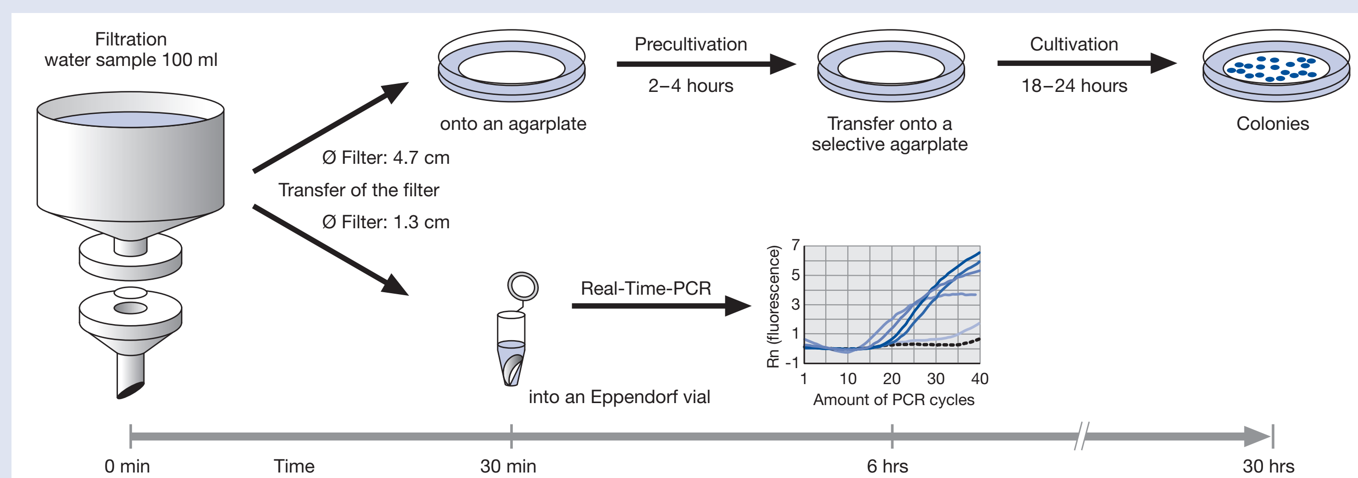

Fig. 1: Individual steps and time requirements for the culturing method (top) and the newly developed PCR method (bottom).

teristic for *E. coli* are replicated in a temperature cycling process. A fluorescent dye which stains double stranded DNA makes the synthesized DNA fragments visible. In the "real-time PCR method" that we use, fluorescence is measured in real time and recorded by a computer. The result is the number of thermal cycles required to reach a detectable fluorescence signal. With respect to a particular water sample, this means that the more *E. coli* cells (i.e., the more *E. coli* DNA) were initially present in the sample, the fewer thermal cycles are needed before the fluorescence reaches the detection level. A crucial element of our PCR method is the pretreatment of the sample with the enzyme DNAse, which removes any free DNA that might be present due to dead bacteria, thus preventing false positive results.

The Difference becomes Apparent

In order to test the agreement between the new PCR method and the traditional culturing method, we inoculated drinking water in the laboratory with *E. coli* cells and stored the water at 4 °C. Over the period of one month, we withdrew samples and analyzed them using the two methods. At the beginning of the experiment (t = 0), both methods detected bacteria (Fig. 2A + B). After that, the number of bacteria detected by the culturing method decreased rapidly. We expected therefore that the number of temperature cycles observed in our PCR method would increase. However, this was not the case. Even after one month, the PCR method still detected the presence of *E. coli*. Apparently, the cells were no longer able to grow on the culture media plates.

Cells that can no longer be cultured but can still be detected by other methods are often called "viable but not culturable" (VBNC). There is a controversy among microbiologists as to whether the detection of such VBNC stages is relevant in the case of the *E. coli* indicator organism or pathogenic organisms. Some studies suggest that such cells can be reactivated or that they can become infectious, respectively [4]; other reports indicate the opposite [5, 6]. Based on our results, we suggest that the cells die continuously, and that the rate at which the cells die depends on the "preconditioning" of the *E. coli* culture.

A Proven Method is Difficult to Replace

This simple experiment shows that the two methods do not lead to the same interpretation and that the PCR method used here cannot replace the culturing method. The principles on which the two methods are based, i.e., the ability to replicate in the culturing method and the detection of specific DNA fragments in the PCR method, must be too different from one another. This case illustrates how strongly the thresholds set for microbial parameters depend on the analytical methods employed.

There have been proposals in the literature to perform a short culturing period of a few hours before using the PCR method, which does improve the agreement between the PCR method and the reference method [7]. In general, there are discussions ongoing and proposals for standards published or in progress in order to normalize the validation of alternative methods in the future [8]. The PCR method presented here could probably be established as a "new" method. This would require, however, that we are able to determine what specific physiological stages of *E. coli* are being detected. For the time being, our new PCR method appears to be applicable rather to research than to the routine monitoring of drinking water.

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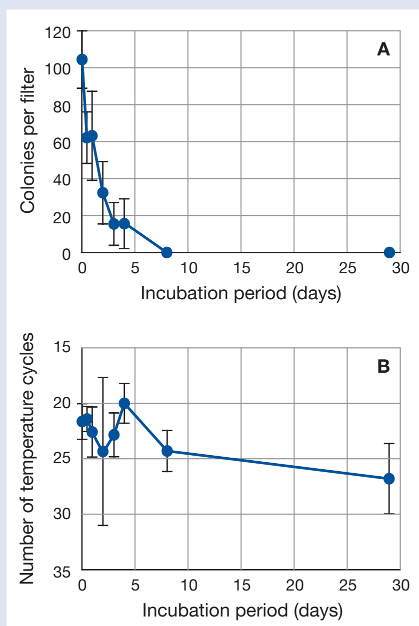

Fig. 2: Results of *E. coli* determinations by the culturing method (A) and the PCR method (B) in artificially contaminated drinking water.
All tests included determinations in non-contaminated water; culturing method: 0 colonies; PCR method: detection level of fluorescence not reached after 40 temperature cycles.

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