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5. Incubation at 44 ℃ as a test for faecal coli

Clegg LFL, Sherwood HP. J Hyg 1939; **39**: 361–374

AN APPRECIATION BY PAUL HUNTER

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Introduction

The nature and frequency of waterborne disease changed substantially during the course of the 20th century [1]. In the early decades of the century, the primary diagnosed cause of waterborne disease in both the United Kingdom and the United States was typhoid fever. It was well known at that time that the primary source of infection was faecal pollution of drinking water. It was against this background that methods for detecting faecal pollution were sought.

It was probably Eijkman in 1904 who first used *E. coli* as a measure for faecal pollution [2]. By the mid-1930s faecal coliforms had become established as the primary indicator of faecal pollution. However, their identification required the performance of a number of confirmatory tests which added to the complexity and cost of testing both water and shellfish. There was a clear need for a single test to differentiate *E. coli* from other coliforms to simplify and speed up the processing of specimens.

It had been know for some time that *E. coli* was able to grow at higher temperatures. However, the value of incubation at elevated temperatures was still open to considerable debate. In part the conflicting results were due to the different temperatures used. Eijkman and Perry preferred incubation at 46 °C, the Metropolitan Water Board preferred incubation at 42 °C and Wilson used 44 °C [3]. A further likely source of unreliability was the difficulty in maintaining temperatures within narrow limits [3].

The paper

The paper chosen for discussion is the one by Clegg and Sherwood [3]. The main focus of this work was the potential use of culture with acid and gas production at 44 °C in MacConkey's broth as a method for detection and/or confirmation of *E. coli* in samples of mussels. They suggested that an organism growing at 44 °C and producing acid and gas was *E. coli* and that primary culture at this temperature would enable a combined isolation and identification process that would not require subsequent confirmation.

This paper is a remarkable example of the pioneering science done at that time. In order to reach their conclusions, the authors had to conduct an exhaustive series of experiments comparing growth at different temperatures. However, in order to do this they first had to design and build a mechanism for maintaining the temperature within a water-bath within very close limits $\pm 0{\cdot}1$ °C.

The authors went onto conduct a huge number of individual experiments on some 522 strains incubated at 37, 41, 42, 43, 44, 45 and 46 °C. They found that citrate-negative lactose fermenters continued to produce acid and gas up to 44 °C, but that above that temperature acid and gas production declined rapidly. By contrast acid and gas production by citrate-positive lactose fermenters declined as the temperature was increased above 37 °C so that very few produced acid and gas at 44 °C. There were 14 citrate-negative organisms that did not produce acid and gas at 44 °C and four citrate-positive organisms that did produce acid and gas at this temperature.

These 18 strains were further identified and the authors concluded that in only two cases did the results produced by their method conflict with the faecal significance of the isolate.

The authors felt confident in recommending the use of culture at 44 °C as a primary isolation and identification system that did not require confirmatory

testing. It is interesting that the authors made it clear that they were not suggesting that their proposed method would be of similar value in water examination. However, it is in regard to water testing that this paper has had most impact on laboratory methodology.

Current value

The impact of this paper on water and shellfish microbiology is immense. The use of 44 °C as the temperature for isolation of *E. coli* has become standard in water microbiology over the 60 years since this paper was published. This elevated temperature is used for both membrane and multiple tube methods for primary incubation in order to exclude many non-faecal coliforms from further analysis.

However, the authors' hope that the approach could remove the need for confirmatory testing has not been achieved. In their own work they demonstrated that some coliforms, other than *E. coli*, also grew at this temperature. This led to the use of the term faecal coliform (more properly thermotolerant coliform) to include all members of the Enterobacteriaceae that fermented lactose with the production of acid and gas at 44 °C. Indeed the term faecal coliform was included in both European and UK legislation as standard indicators of water quality [4–6].

It is only recently that water microbiologists have begun to move away from the basic approach suggested by Clegg and Sherwood [3]. This has come about primarily because of the realization that *E. coli*, but not the other thermotolerant coliforms, has direct relevance to public health risk [7, 8]. It is no longer acceptable to use faecal coliforms as an indicator of faecal contamination when many thermotolerant species are not necessarily faecal in origin. Consequently new European and UK legislation has dropped the term faecal coliform in favour of *E. coli* as the standard indicator of faecal contamination [9, 10]. Indeed the choice of *E. coli* as the standard has allowed new tests, which are based on detection of the enzyme β-glucuronidase, to be developed [7]. These new tests do not even require culture at the elevated temperature. On a final point, there is also some evidence that faecal streptococci in drinking water are more closely associated with risk of gastrointestinal illness than *E. coli* [11].

Nevertheless, despite these technical developments and recent changes in emphasis, primary incubation of water and shellfish samples for *E. coli* usually still proceeds at 44 °C, the temperature shown to be the most effective for this task by Clegg and Sherwood [3].

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