

Colifast Micro Detector to detect coliforms in water

Monitoring recreational/surface water and waste water is subject to special requirements in order to best protect the environment and public health. It is beneficial to have a rapid field test which can provide early warning of bacterial contamination, instead of waiting for the 24-48 hour turnaround for results from a laboratory.



The Colifast Microdetector (CMD – the Turner Designs *Aquafluor™*) is a battery-driven handheld fluorescent analyser that can be used as an analyser for various methods. The Colifast method used in this trial is based on the enzymatic reaction of β -D-galactosidase. This enzyme is present in coliforms and cleaves the substrate 4-methylumbelliferyl- β -D-galactoside (MU-gal) in the Colifast 6 media leading to the fluorescent end-product methylumbelliferone (MU). Changes in the fluorescence level during the short lag phase (before bacterial growth) were measured by the Colifast Microdetector and the results were compared with the results from a standard culturing method for thermotolerant coliforms.

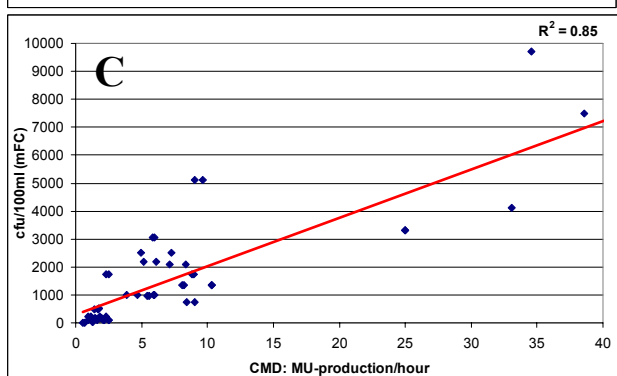
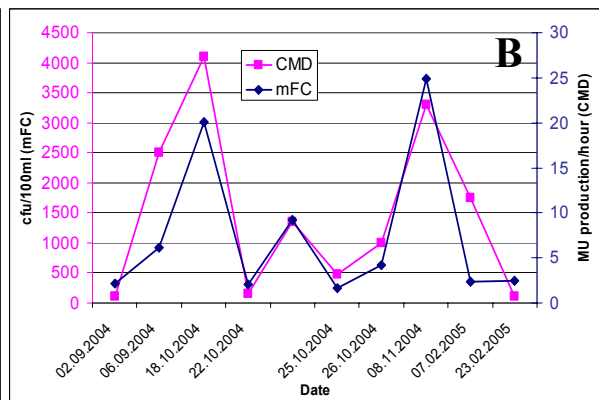
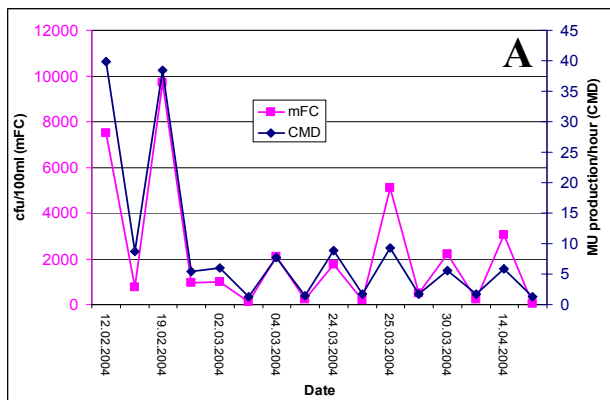
Based on the good quality of the preliminary results and feedback from potential users, the CMD Field Kit, was developed and launched in 2005. This kit contains the CMD, an incubator, test-tubes pre-filled with Colifast 6 media, cuvettes, developer, adapters, and disposables needed for field analysis. The CMD Field Kit turned out to be a useful tool for both international aid work agencies and municipalities. A number of results from these field applications were compared with results from analysis performed at laboratories. An overview of the application areas for the CMD and the CMD Field Kit is shown in the Table below.

User	CMD/Kit	Sample	Source	Method	Benefits
Water works	CMD	Water	River/raw water, In-process, waste water, finished water*	TTD, P/A, MUP	Improved process control, rapid results, pinpoint contamination
Aid work	Kit	Water	Raw water sources, finished/drinking water*	MUP, P/A	Rapid results – select source, treatment control, easy to use, enhanced safety
Industry	CMD/Kit	Water/milk	Process, waste, product	TTD, P/A	Improved process control, product safety, rapid results, save time
Commercial hotels, ships, offshore, etc	Kit	Water	Water tank, well, supply*	P/A	Improved process control/safety, easy to use
Community Services	CMD/Kit	Water	Rivers, lakes, sea, bathing water, raw water, drinking water	TTD, P/A, MUP	Environmental surveillance, pinpoint contamination, enhanced safety, easy to use
Public Health Authorities	CMD/Kit	Water/milk	Samples from milk and water suppliers	P/A	Rapid results, improved quality control

TTD: 4 – 12 hours P/A: 8 – 11 hours MUP: 2 hours
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Results

The development of fluorescence analysed by the Colifast Microdetector (CMD) and the calculated slope (MU/hour) showed good correlation with traditional culturing methods (mFC). 17 water samples, further diluted to a total of 27 samples were analysed at the Colifast laboratory. The bacterial level in the samples varied from 30 cfu/100ml to 9700 cfu/100ml. All measured differences in bacterial level, based on mFC counts, were predicted in advance by the 2 hour CMD test. The time-to-detect (TTD) with this field method at these bacterial levels will be 2 hours; with sample preparation time the total time-to-result (TTR) will still be less than 3 hours. The results also showed good linearity ($R^2=0.85$) as shown in the Figures below. This level of linearity was also seen in the work presented in studies in the UK and Sweden in which approximately 300 samples had been analysed from different locations, which explains why such a small number of samples was taken in this study, and which demonstrates the utility of the method. Even with traditional culturing methods the variations between duplicate tests can be significant. For example, a range of counts of at least 1 log is reasonable at relatively low counts for environmental water samples. The hydrolysis rate of MUGal could thus be estimated more accurately and quickly than the content of indicator bacteria with traditional culturing methods. One should bear in mind that the results may be altered by MUGal activity from viable but non-culturable bacteria in the water sample. In this regard, it is recommended to calibrate the method for each site.



- A.** Øverland river: mFC counts shown as cfu/100ml and the calculated slope values (MU-production/hour) based on CMD results.
- B.** Lysaker river: mFC counts shown as cfu/100ml and the calculated slope values (MU-production/hour) based on CMD results.
- C.** Linearity plot for CMD (2h) results versus mFC results. $R^2 = 0.85$

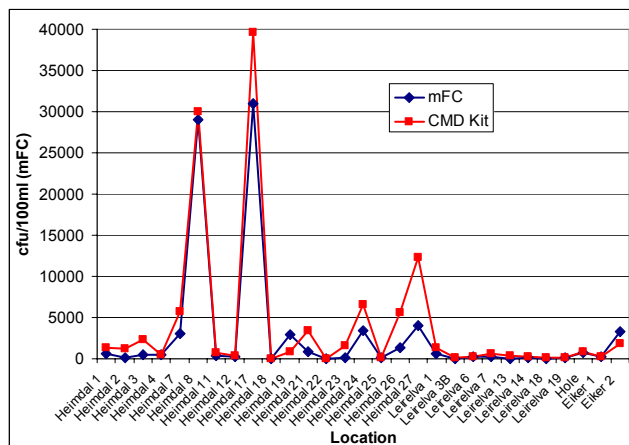
Based on the results from the trial on fresh water samples a table of the CMD slope related to bacterial level can be made (see table below). In this table there is a linear slope where 1 equals 200 cfu/100ml (for slope values over 2.5). This can be used initially, and then revised as needed by calibration at the site for regional or seasonal differences in the coliform population.

CMD slope (MU-production/hour)	cfu/100 ml
< 2.5	~ 0
2.5	500
5	1000
10	2 000
20	4 000
40	8 000
80	16 000

Table 2. CMD slope correlated to bacterial level.

The CMD Field Kit was evaluated by three municipalities in Norway and by local engineers with no microbial or laboratory experience. To further simplify the procedure, the CMD Field Kit testing was done without parallels. Also, some of the results were calculated after only 45 minutes test time. This may bias the accuracy of the results. However no significant difference was found between parallels, and little difference between 45 minutes and 2 hour and 15 minutes results in the preliminary trial. The operators also experienced that contaminated samples were indicated by high fluorescence readings after 15 minutes.

29 CMD Field Kit results from various locations were compared to results from laboratory analysis of collected samples (see figure below). The calculated bacterial number showed good correlation with traditional culturing methods ($R^2=0.97$).



Analysed river water samples from several remote locations in Norway. mFC counts versus calculated bacterial level based on CMD Field Kit results.

During the summer months of 2005 the method was also tested on 60 sea/bathing water samples. Problems with enzymatic methods have been reported on saltwater samples. Saltwater added to saline media may cause altered bacterial activity and also some marine algae may interfere with the measured enzymatic activity. The CMD results from the sea water samples did show less accuracy when compared to results from traditional culturing method (results not shown). Based on these results the rapid CMD method is not recommended for sea water samples.

Summary

The results from the CMD MU-production/hour tests have shown that CMD can give accurate results (TTD) after 2 hours, and TTR <3 hours after the sample is taken. The method is essentially a field-enabled chemical assay with three steps requiring a low skill level:

- add sample to vial
- pipette a sub-sample at the reading time
- add alkaline developer to the sub-sample and read the result.

There are no manipulations such as membrane filtration which are complicated, or which require special techniques in the field. This means a useful field tool with great operational value. Moreover, no other available tests can produce results so quickly. Due to greater variability at low bacterial levels this method should preferably be used on samples containing >500 cfu/100ml. Lower levels of coliforms can easily be detected by simply extending the incubation times.

In a number of situations operational measures could be taken at an early stage, e.g. actions against contamination, closing raw water intakes, etc. The small size, low weight and a minimum of ancillary equipment, i.e., only a pipette and incubator, also allow testing at remote locations. The all inclusive CMD Field Kit enables personnel to do rapid, accurate and uncomplicated testing at site. Also, an early indication of contamination (15 minutes) gives the ability to screen and locate contamination sources very quickly.

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