

Technical Note No. 8 (rev 8.3)**COLIFAST MICRODETECTOR (CMD) RAPID (2 h 15 min) FIELD COLIFORM TEST**

Evaluation of a MU-production (2 h 15 min) Method for Detection of Thermotolerant Coliforms in Water using Colifast Microdetector

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Introduction

In February 2004 Colifast® AS started a project to further evaluate the existing Colifast Microdetector for its performance in rapid microbial testing of recreational/raw water and waste water quality. The project further evolved to development of a all included field kit. The resulting method should be well suited for surface water with varying faecal contamination, and it proved to be a flexible and field-capable early warning system.

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

The preliminary analyses were carried out at Colifast's laboratory at Lysaker. The water samples were collected from the Øverlandselven and the Lysakerelven, two separate streams passing through suburban areas outside Oslo. The Colifast Microdetector (CMD) 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) (1, 2). 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 (3).

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. All this was gathered in a plastic case. It can operate on a 12 volts power supply and can be connected to the lighter plug in a car. The CMD Field Kit turned out to be a useful tool for both aid work agencies and Norwegian 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 Table 1.

User	CMD/Kit	Sample	Source	Method	Benefits
Water works	CMD	Water	River/raw water, Process, waste water, finished water*	TTD, P/A, MUP	Increased control, rapid results, pinpoint contamination
Aid work	Kit	Water	Raw water sources, finished/drinking water*	MUP, P/A	Rapid results – select source, treatment control, handy-easy to use, increased safety
Industry	CMD/Kit	Water/milk	Process, waste, product	TTD, P/A	Increased control, product safety, rapid results, save time
Commercial hotels, ships, offshore, etc	Kit	Water	Water tank, well, supply*	P/A	Increased control/safety, handy-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, increased safety, handy-easy to use
Public Health Authorities	CMD/Kit	Water/milk	Samples from milk and water suppliers	P/A	Rapid results, increased control

Table 1. CMD applications. * *Sample may need chloride neutralization*

Detection time

TTD: 4 – 12 hours P/A: 8 – 11 hours MUP: 2 hours
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The following is a summary of the results up to and including December 2005.

Materials and methods

Sampling

A series of surface water samples were collected from Øverland river and Lysaker river in Bærum, Norway. The samples were periodically collected from February 2004 to December 2005. The samples were collected in sterile bottles, refrigerated and assayed within 5 hours.

Several samples from surface water and waste water at different locations in Norway were analyzed at site with the CMD Field Kit and also collected in clean bottles, refrigerated and assayed within 12 hours at a laboratory.

Media

mFC (Difco, USA) agar plates were prepared according to manufacturers instructions. Colifast 6 was prepared by adding a vial of media (13g) to 100ml of distilled water, stirring to mix, then adding 400ml of boiling distilled water, and stirring to mix. (Pre-filled vials containing portions of Colifast 6 media, e.g., 10 ml, has a shelf life of at least 6 months, greatly simplifying field work.)

Sample preparation and procedure, mFC

Water samples (0.1 and 1.0 ml) were filtered through sterile 0.45µm membrane filters (Millipore, USA) in duplicate using standard membrane filtration technique. The membrane filters were placed on 47mm Petri dishes containing mFC agar. After 24 hours incubation at 44°C, the plates were read and blue colonies were counted and registered as thermotolerant coliforms. Similar method was used by Næringsmiddelkontrollen in Trondheim for analysis of collected samples.

Sample preparation and procedure, Colifast Microdetector

10 ml water samples were added by pipette to duplicate vials pre-filled with 10 ml of Colifast 6 medium, and placed in an incubator at 44°C. For tests performed at site; 10 ml water sample was added by a syringe to a single pre-filled tube and incubated at 44°C. A 3 ml sub-sample was taken every 30 minutes after 15 minutes warm-up, mixed with 0.1ml 0.5M NaOH and measured with the Colifast Microdetector. The relative fluorescence values (rfu) were registered for every sub-sample and the increase in rfu was calculated and registered as ppb MU/hour (slope).

Statistical evaluation

The average count of two parallel samples of mFC agar in the 10-100cfu/plate range was used to make a regression plot of the CMD results versus mFC plate count results.

Results and discussion

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 (Figure 1A). 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 Figure 1B. This level of linearity was also seen in the work presented in studies in the UK and Sweden (1,5) 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. Another UK study (6) reports detection specific for *E.coli* at these levels of contamination in about 4 hours. 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 (4). The hydrolysis rate of MUGal could thus be estimated more

accurately and quickly than the content of indicator bacteria with traditional culturing methods. But 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.

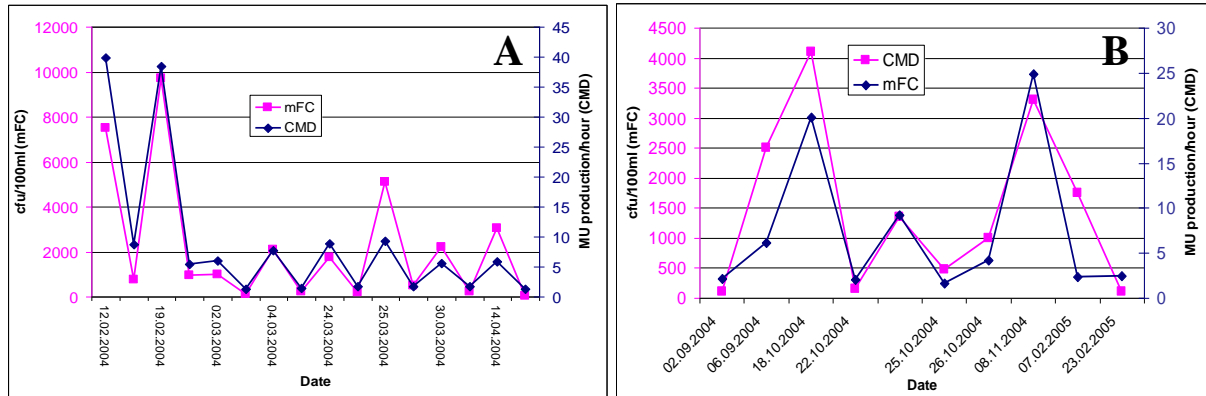


Figure 1. A. Øverland river: mFC counts shown as cfu/100ml and the calculated slope values (MU-production/hour) based on CMD results.
B. Lysaker river: mFC counts shown as cfu/100ml and the calculated slope values (MU-production/hour) based on CMD results.

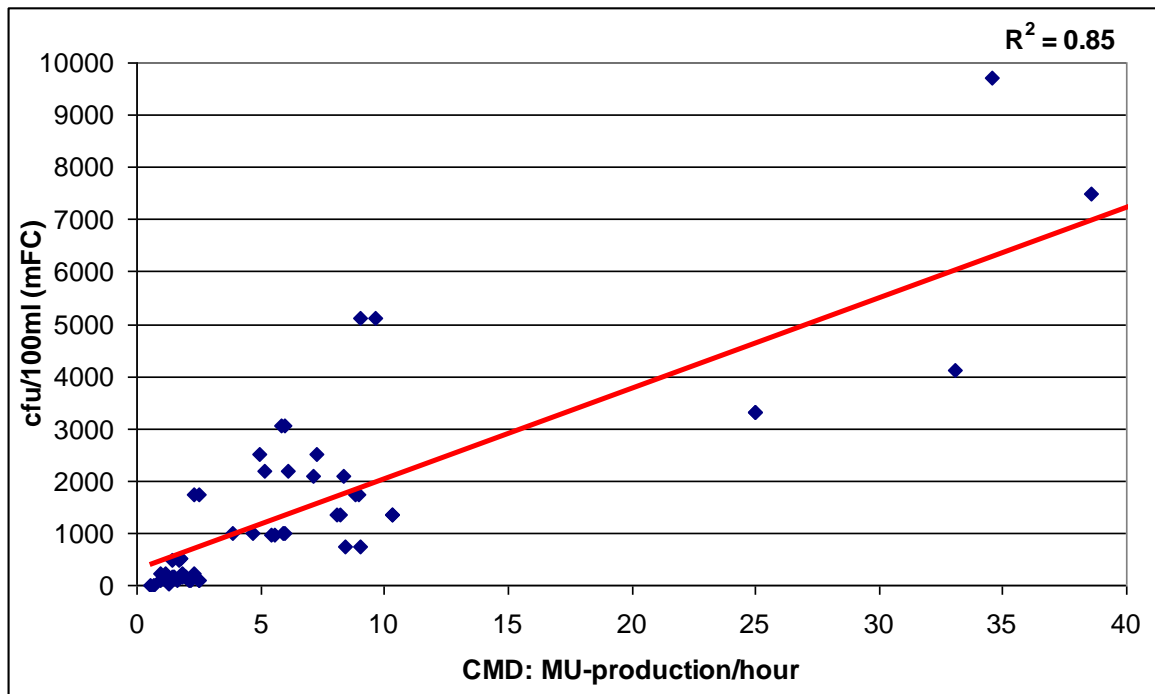


Figure 2. Linearity plot for total CMD results versus mFC results. $R^2 = 0.85$.

Based on the results from the trial on fresh water samples a table of the CMD slope related to bacterial level can be made (Table 2). 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 correlation table from the preliminary laboratory study was used as a guideline for field testing with the CMD Field Kit. And the bacterial number was calculated directly by the user. The CMD Field Kit was primarily operated 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 there was not found any significant difference 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 reading after 15 minutes.

29 CMD Field Kit results from various locations were compared to results from laboratory analysis of collected samples (Figure 3). The calculated bacterial number showed good correlation with traditional culturing methods ($R^2=0.97$).

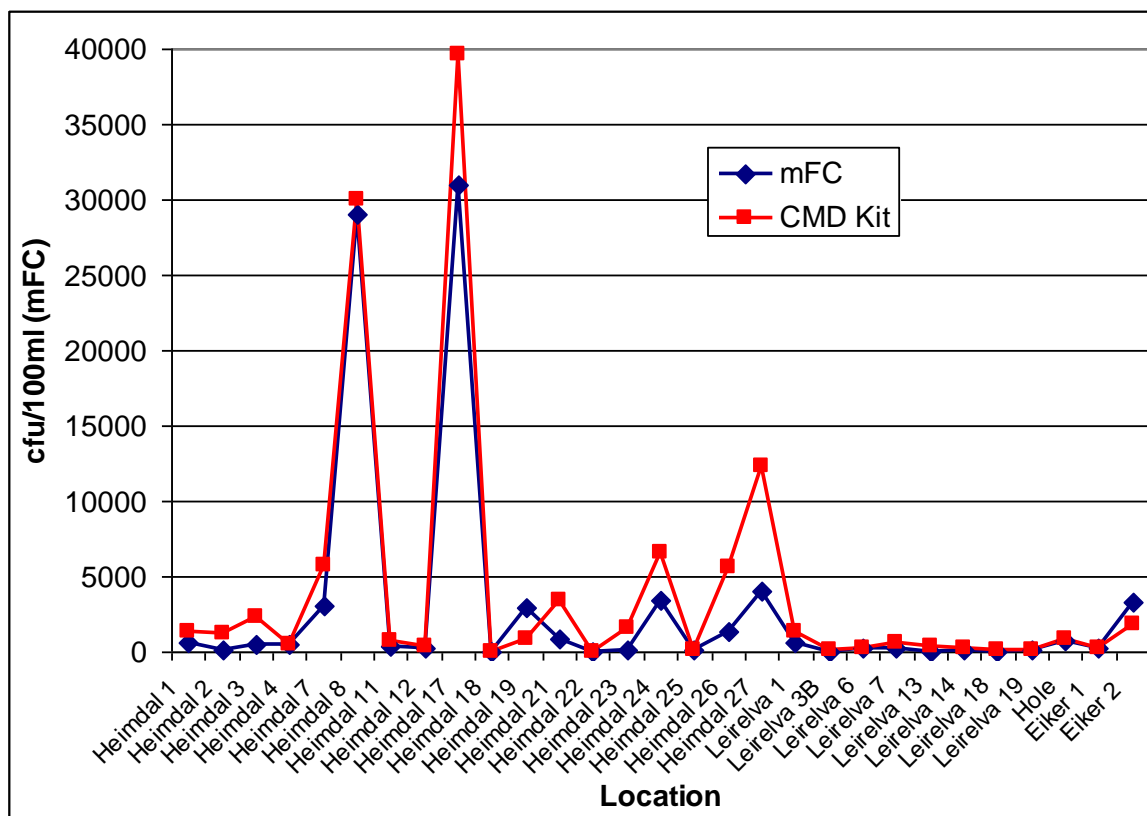


Figure 1. A. Analysed river water samples from several remote locations in Norway. mFC counts versus calculated bacterial level based on CMD Field Kit results.

During the summer months of 2005 the method was in addition tested on 60 sea/bathing water samples. Problems with enzymatic methods have been reported on salt-water samples (7,8). Salt-water 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.

Conclusions

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. An example of this is given in reference 9.

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, is also be a benefit allowing testing at remote locations. The all inclusive CMD Field Kit enables personnel to do rapid, accurate and uncomplicated testing at site (10). Also, an early indication of contamination (15 minutes) gives the ability to screen and locate contamination sources very quickly.

A list of typical customers and their applications is shown in Table 3.

Customer	CMD/Kit	Sample Source	Detection	Method
Thames Water, water works, UK	CMD	River water, process water, waste water	Coliforms, <i>E.coli</i>	TTD, P/A, MUP
Norrvatten, water works, Sweden	CMD	River water, lake water, well water, process water, drinking water	Coliforms, <i>E.coli</i> , TVO*	TTD, P/A, MUP
Scan-Water, aid work agency, Norway	Kit	River water, well water, drinking water	Coliforms	P/A, MUP
UMB, University, Norway	Kit	River water, lake water, drinking water	Coliforms	P/A, MUP
University?, China	CMD	River water?, waste water, lake water?	Coliforms, <i>E.coli</i>	TTD, P/A, MUP
Nedre Eiker, County, Norway	Kit	River water, waste water	Coliforms	MUP
Oppegaard, County, Norway	Kit	River water, waste water	Coliforms	P/A, MUP
Hole, County, Norway	Kit	River water, lake water	Coliforms	P/A, MUP
Trondheim, County, Norway	Kit	River water	Coliforms	MUP
Arendal, County, Norway	Kit	River water, waste water, drinking water	Coliforms	P/A, MUP
Lindesnes, County, Norway	Kit	River water, waste water	Coliforms	MUP

Table 3. Customers and applications. * TVO: Total Viable Organisms

References

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