ColiMinder
rapid microbiology by VWM SOLUTIONS

Technical Review for UNICEF

Excerpt

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THE COLIMINDER MONITORING DRINKING WATER PRODUCTION IN OBERWEIDEN AUSTRIA

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Introduction

VWMS GmbH, an Austrian SME, develops and manufactures fully automated stand-alone measurement systems named **ColiMinder®** for microbiological water quality using a rapid measurement approach based on enzymatic activity. The amount of enzyme in a sample, determined via its activity, is used as a proportional metric for the presence of microorganisms.

Escherichia coli is the most common fecal indicator and qualitative evidence of β-glucuronidase activity is an established method of detection. This approach is also used by the cultivation-based reference method. Our vision is to *directly* link β-glucuronidase activity with fecal contamination and microbiological risk of the presence of pathogens. Due to technological advances, it has become feasible to quantitatively detect β-glucuronidase activity in an automated and time-saving process, and thus the indirect route via statistical enumeration of culturable *E. coli* CFU can be circumvented.

1 Key functions

ColiMinder measures the enzymatic activity of certain enzymes. Depending on the enzymatic assay, the following target organisms can be detected:

- β-glucuronidase: *E. coli*
- β-glucosidase: Enterococci
- Alkaline phosphatase: total activity
- *β-galactosidase: Coliforms (in development)*

The respective reagents for each assay are provided by VWMS GmbH in kits of 250, 500, or 1,000 measurements.

The measurement is carried out in the liquid phase and a single full working cycle takes approx. 30 minutes including cleaning, while **time to quantitative result is 15 minutes**. 10-15 ml of sample in total is required for a single determination. The measurement process is comprised of different phases: It is initiated by the automatic dosage of reagents to the sample within a measurement chamber, followed by heating of the sample to a defined temperature, and finally measuring the formation of fluorescent product due to enzymatic activity (continuous rate determination). Each device is calibrated to convert the rate to enzymatic activity in Units/100 ml, based on the respective unit definition and reference method. In certain intervals, 'blank' measurements with clean deionized water as a sample are performed. Occasionally indicated re-calibrations of the device hardware are run autonomously.

The measurement process and the cleaning procedure are performed automatically by the device. ColiMinder is controlled with a Graphical User Interface on a tablet PC, smartphone or any computer which is connected to the machine via Wi-Fi, LAN or remotely via internet connection through internal mobile network modem. ColiMinder can be operated in different measurement modes: Single measurements (triggered by the user), measurements in defined time intervals, or continuous measurements for almost real-time monitoring of water sources. The necessary consumables (Reagents A and B, wash-solution concentrate and rinsing water) are sufficient for up to 1,000 measurements (ColiMinder® Mobile: 250 measurements) until a refill is required[1]. ColiMinder is equipped with an internal reagent cooling unit to ensure stability and constant high quality of the inserted reagent batches.

[1] Depending on the device version, the number of consecutive measurements before a manual refill is necessary may differ. See Table 1 for more information.

ColiMinder is currently offered in four versions (Figure 1):

- ColiMinder Industrial Standard CMI-02: Intended for stationary operation at representative sampling sites.
- ColiMinder Industrial Low Energy version CMI-02-LE
- ColiMinder Emergency Response Unit (ERU): Industrial LE, mounted on a rack with all necessary containers for rinsing water and waste, can be carried by two persons and operated on a car or boat due to its flexible power source.
- ColiMinder Mobile Unit CMM-02: Portable in a PELI- case with all necessary containers, can be carried by a single person, designed to be used in remote areas.

Table 1 summarizes some key specifications of these versions.

Future R&D efforts at VWMS GmbH are dedicated to developing a more sophisticated and smaller hand-held version for even simpler and more flexible use.

For any of the three described use cases in the TPP, different versions of the ColiMinder are recommended:

I. Use case A – Household Surveys and MICS
Recommendation: ColiMinder Mobile CMM-02
Reason why: The Mobile version is easy to carry and enables the household survey team to walk from door to door and realize measurements directly in the household together with the interviewed persons. The short time to result (15 minutes) enables *in-situ* communication and appropriate action to the household members.

II. Use case B – Community-led monitoring, Behavior Change, and Emergency preparedness
Recommendation: ColiMinder Emergency Response Unit CMI-02-ERU
Reason why: The ERU comprises of a ColiMinder LE (low energy) assembled on a rack with all necessary equipment on board. It is dedicated to being used in cars/ on boats, measuring while moving from one sampling point to another. Its capacity for deployment in different applications – mobile or stationery – together with the 15 minutes time to result make it the best and most flexible solution for this use case.

III. Use case C – Regulatory oversight & Surveillance for onsite Testing
Recommendation: ColiMinder Emergency Response Unit CMI-02-ERU
Reason why: The ERU comprises of a ColiMinder LE (low energy) assembled on a rack with all necessary equipment on board. It is dedicated to being used in cars/ on boats, measuring while moving from one sampling point to another. Its capacity for deployment in different applications – mobile or stationery – together with the 15 minutes time to result and its capacity for permanent monitoring installations make it the best and most flexible solution for this use case.

TABLE 1: SPECIFICATIONS OF DIFFERENT COLIMINDER VERSIONS. MAX. NUMBER OF WASH SOLUTIONS: A SINGLE WASH SOLUTION IS SUFFICIENT FOR STANDARD APPLICATIONS. ADDITIONAL WASH SOLUTION FORMULATIONS ARE OPTIONALLY AVAILABLE FOR SPECIAL APPLICATIONS (E.G., WASTEWATER SEWAGE).

	Industrial Standard	**Low Energy**	**ERU – Emergency Response Unit**	**Mobile**
Measurement time (min)	15	15	15	15
Full working cycle duration incl. automated cleaning (min)	30	30	30	30
Measurement frequency	56 per day (up to 80)	56 per day (up to 80)	56 per day (up to 80)	56 per day[2] (up to 80)
Reagent capacity	1,000 measurements	1,000 measurements	1,000 measurements	250 measurements[3]
Max. number of wash solutions	3	3	3	1
No. of sample inlets	2	2	10 (2)	1
Housing	Powder-coated aluminum	Powder-coated aluminum	Powder-coated aluminum	PELI Mod. 1440

FIGURE 1: COLIMINDER® VERSIONS. LEFT: COLIMINDER INDUSTRIAL STANDARD AND LOW ENERGY VERSION. MIDDLE: COLIMINDER EMERGENCY RESPONSE UNIT ERU. RIGHT: COLIMINDER MOBILE.

2 Power requirements

TABLE 2: POWER CONSUMPTION AND POWER SUPPLY OPTIONS FOR COLIMINDER® VERSIONS

	Industrial Standard	**Low Energy**	**ERU – Emergency Response Unit**	**Mobile**
Power consumption	86-264 V AC 150 W	12 V DC 35W (max 55 W)	12 V DC 35W (max 55 W)	12 V DC 35W (max 55 W)
Power supply options	• AC	• External batteries • Car/boat 12 V DC • Solar power • External AC-DC converter	• Internal batteries • External batteries • Car/boat 12 V DC • Solar power • External AC-DC converter	• Internal batteries • External batteries • Car/boat 12 V DC • Solar power • External AC-DC converter

[2] 12 measurements with a single battery charge

[3] 30 measurements until a rinsing water refill is required

3 Safety requirements

I. **ColiMinder® device:** The housing of the ColiMinder® is protection class IP65. There are no further safety requirements except handling with care as for any electric device. Risk assessment can be provided upon request.
II. **Reagents**: Non-hazardous and non-toxic. Please see attached MSDS. No hazardous material according to ADR.
III. **Waste**: The waste resulting from the measurement process is less biologically contaminated than the sample taken and contains no hazardous chemicals.

4 Equipment dimensions

Dimensions and weight of ColiMinder versions are presented in Table 3. For Industrial Standard and LE versions, additional space for rinsing water and wash solution canisters (and if required on site, a waste container) must be considered. ERU and Mobile versions are equipped with all necessary containers.

TABLE 3: DIMENSIONS AND WEIGHT OF COLIMINDER® VERSIONS

	Industrial Standard	**Low Energy**	**ERU – Emergency Response Unit**	**Mobile**
Dimensions W x H x D (cm)	50 x 50 x 21	50 x 50 x 21	56 x 56 x 64	50 x 30.5 x 45.7
Weight	17 kg	15 kg	Approx. 35 kg Incl. 40 Ah battery	16 kg Incl. 15 Ah battery

5 Performance: Sensitivity and Specificity

Sensitivity and specificity for the detection of *E. coli* with a real-time enzymatic approach have different prerequisites than for cultivation-based methods.

In cultivation-based methods, the cultivation step is utilized for:

- Selective enrichment of the target organism (increase of sensitivity and specificity)
- Induction of the expression of the enzymatic activity which is used for detection (increase of sensitivity)

Due to the lack of a cultivation step, the situation with rapid enzymatic methods is more complicated: Sensitivity not only depends on the measurement parameters (technical and biochemical setup of the measurement), but also on biological parameters, which cannot be influenced in the case of rapid methods (otherwise, these are ruled out mostly by cultivation and induction). The sensitivity to detect *E. coli* differs with the sample conditions. Because the number of enzyme molecules per cell can vary over time due to its dependence on the metabolic state of a cell, there is the possibility for changes in proportionality. However, since the majority of cells in a certain environment experience the same conditions, their metabolism is generally very uniform, and results from similar sample matrices are highly reproducible and comparable. Nevertheless, in very specific cases, e.g., in a direct comparison between a sample with well-kept laboratory organisms and a sample of natural water with organisms removed from their natural habitat and struggling for survival, the results should be interpreted with caution. A further elaboration and experimental evidence on this matter is included in Section 9 ‘Level of detection’.

The specificity for the detection of *E. coli* is lower with rapid methods compared to cultivation-based methods due to the lack of selective enrichment (suppression of false positives). Rapid enzymatic methods detect all β-glucuronidase activity in the sample, regardless of its source. However, with respect to the detection of fecal contamination, this is not a drawback at all, since other species of gut bacteria also possess β-glucuronidases [1]. In this context, false-positives regarding the detection of *E. coli* are not necessarily false-positives regarding the risk of contamination with pathogens. Furthermore, the otherwise inaccessible viable-but-non-culturable (VBNC) organisms can be detected using rapid methods. Rapid microbiological measurements via enzymatic activity are sensitive enough to detect the products of enzymatic reactions in the sample without the need of signal amplification by cultivation of the target organism. Therefore, both culturable and non-culturable organisms are accounted for with the same sensitivity. Moreover, the signal in rapid enzymatic measurements is directly proportional to the amount of enzyme in a sample, independent of the physical association of target cells. Methods producing results in Colony Forming Units (CFU) or MPN are prone to inaccuracies, since multiple target cells might appear as a single CFU due to agglomerations of cells, or the association of multiple cells with particles or debris.

Summarizing, traditional cultivation-based methods might miss out on a fraction of target organisms due to VBNC organisms (false negatives) and agglomerations of target organisms which only produce one colony, while rapid methods might tend to produce false positives under some circumstances. In terms of risk assessment, the false negatives might be more problematic.

[1] M. Dabek, S. I. McCrae, V. J. Stevens, S. H. Duncan, and P. Louis, "Distribution of β-glucosidase and β-glucuronidase activity and of β-glucuronidase gene gus in human colonic bacteria: β-Glycosidase activity in human gut bacteria," *FEMS Microbiology Ecology*, vol. 66, no. 3, pp. 487–495, Dec. 2008, doi: 10.1111/j.1574-6941.2008.00520.x.

6 Life Span

I. **ColiMinder device:** the instrument's life span is minimum 10 years. The online support package, contained in the "Costs per 1,000 measurements" proposed, includes extended warranty. This incorporates free exchange of spare parts and therefore guarantees unlimited life span.

II. **Reagent**: sensitive reagents are shipped as two components, which are tested to withstand temperatures of 36°C for several weeks. After mixing, shelf life of reagents is several years at -20°C and several months at +8°C for. Inserted in ColiMinder, the reagent is kept below +8°C by the instrument's reagent cooling system.

7 Regulatory Approvals

The Danish EPA awarded an installation for monitoring hospital sewage discharge including the ColiMinder as "Best Available Technology".

Besides that, ColiMinder does not possess regulatory approvals yet, however, there are peer-reviewed studies which investigated the possibilities to evaluate the microbiological risk with ColiMinder. Currently, several ColiMinder devices are deployed by authorities in pilot installations (Paris, Montreal, Marseille, and Tokyo) monitoring recreational waterbodies. A list of peer-reviewed studies is attached in Annex A. Studies can be obtained upon request.

8 Sample Size Characteristic

VWMS' reagents are optimized to ensure a constant and reproducible milieu in enzymatic determinations independent of the sample matrix. Buffering agents and other components minimize the effect of variable pH and sample matrix composition on enzymatic activity across the typical range of surface or drinking waters. Specialized reagent kits are available for determinations in high salinity water (sea water) for β-glucuronidase and β-glucosidase assays. Effects of turbidity and absorbance of the sample are corrected in ColiMinder's algorithm, based on transmission data which is collected in parallel to the fluorescence data. The ColiMinder can measure samples with a turbidity of up to 500 NTU. If required, automatic sample dilution is available. The environmental temperature range for operation is 0°C to +40°C.

9 Level of Detection

Comparison of ColiMinder analyses to a reference method

As already outlined in Section 5 "Performance: Sensitivity and Specificity", the level of detection with respect to *E. coli* depends on the source of a sample. To provide evidence, and to evaluate the analytical results yielded in ColiMinder measurements against a reference method (IDEXX Colilert), experiments with three representative samples were conducted. Results from analyses of serial dilutions of a suspension of an *E. coli* laboratory strain, a natural surface water sample, and drinking water with fecal contamination were compared. For demonstration purposes with ColiMinder measurements two alternative assays were performed: β-glucuronidase (GUS) activity and Alkaline phosphatase (ALP) activity measurements. β-glucuronidase is generally used as a typical marker for *E. coli* and more specific for certain bacteria, whereas Alkaline phosphatase is more abundant among different organisms and therefore reflects the total microbiological activity in a sample. While β-glucuronidase is suited for detection and monitoring of fecal contamination, Alkaline phosphatase offers the possibility to monitor any kind of biological contamination. Enzymatic activity of β-glucuronidase (GUS) is given in milli Modified Fishman Units per 100 ml (mMFU/100 ml). One MFU is the amount of enzyme that releases 1 µg of phenolphthalein from phenolphthalein glucuronide per hour at 37°C (pH 6.8). Alkaline phosphatase (ALP) activity is given in micro Units per 100 ml (µU/100 ml). One Unit of Alkaline phosphatase hydrolyzes 1 µmol of *para*-nitrophenyl phosphate per minute at 37°C (in glycine buffer pH 10.4). The current lower limits of detection are 0.5 mMFU/100 ml (GUS) and 1 µU/100 ml (ALP).

Results and discussion

Evaluation of ColiMinder® measurements of a natural surface water sample against a reference method

Our experience has shown that detection limits in ColiMinder measurements of natural water samples differ substantially from those of laboratory samples. In order to provide further evidence, serial dilutions of water from a small stream were prepared and analyzed with both ColiMinder assays and the reference method (dilutions NW1-NW9, Fig. 4). Again, all three methods show a linear dependency on the dilution factor. Interestingly, at low contamination levels, the reference method reaches its limits, as the results do not allow for a distinction between samples NW8 and NW9 anymore (Fig. 4 C and Table 5). The whole range of ~600-3 MPN/100 ml could be measured with both GUS and ALP.

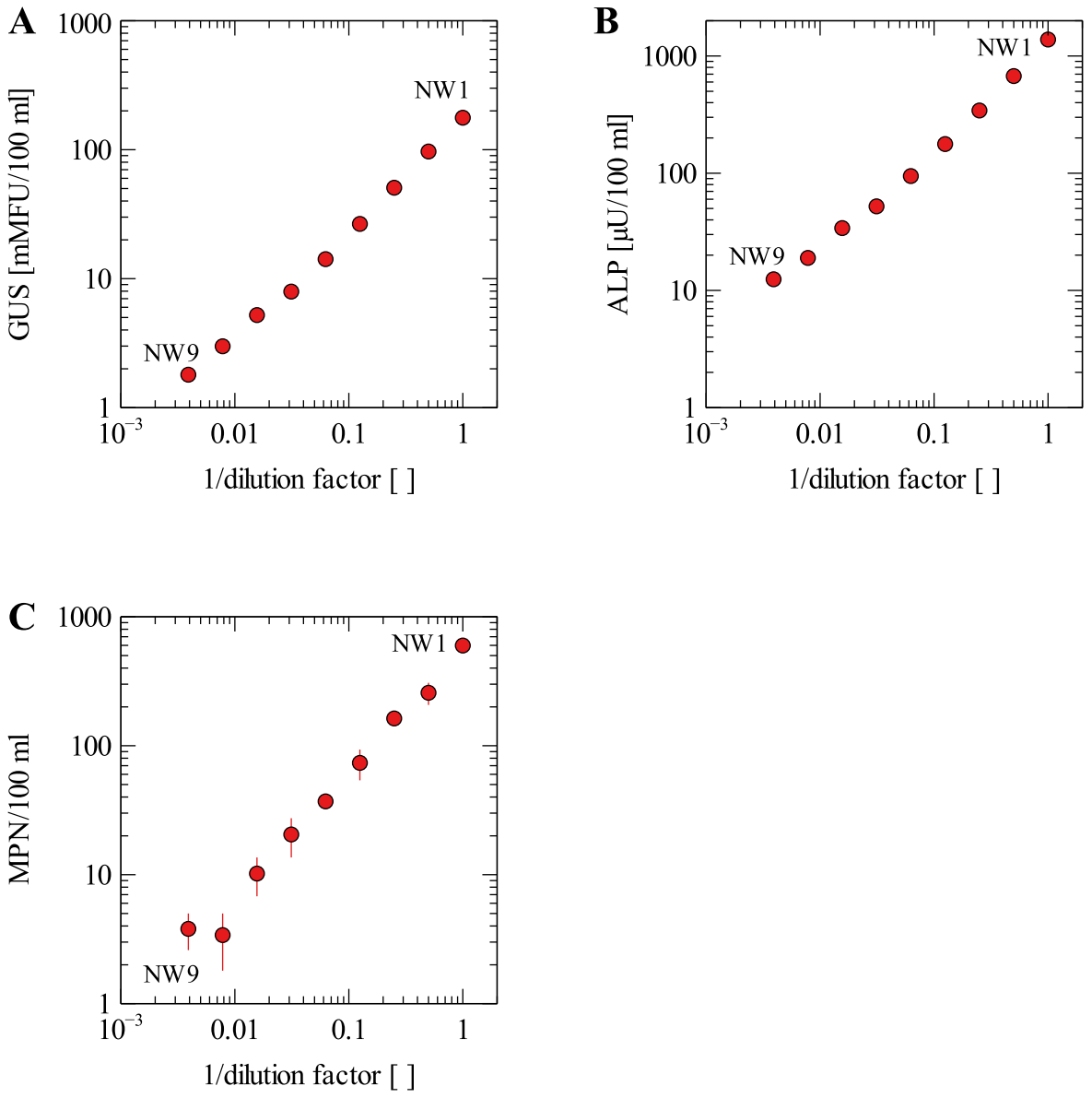

FIGURE 2: ANALYSIS OF SERIAL DILUTIONS OF A NATURAL WATER SAMPLE. A: B-GLUCURONIDASE ACTIVITY (GUS); B: ALKALINE PHOSPHATASE ACTIVITY (ALP); C: IDEXX COLILERT MOST PROBABLE NUMBER (*E. COLI*). RESULTS ARE PLOTTED AGAINST THE INVERSE OF THE DILUTION FACTOR ON A DOUBLE LOGARITHMIC SCALE. ERROR BARS REPRESENT THE STANDARD DEVIATION OF THREE DETERMINATIONS.

The detection limit for GUS and ALP is below a corresponding MPN count of 3.4-3.8 MPN/100 ml (Fig. 5 and Table 5). We hypothesize, that the remarkably lower detection limit with GUS in natural water samples is mainly caused by two effects: 1) expression levels of β-glucuronidase could be increased within this environment, thus the number of molecules/cell and therefore the signal/cell is highly increased, and 2) gain of signal due to β-glucuronidase from organisms other than *E. coli* (other bacteria of mostly fecal origin). In the case of ALP, the presence of other organisms definitely contributes to the signal, which is why the readings for this assay are generally higher for a complex sample.

Both assays with ColiMinder show a decent linear dependency on the contamination level based on the reference method, and even yield results for dilutions, where the reference method reaches its limits. The ratio of *E. coli* MPN and β-glucuronidase activity is ~ 3 MPN/mMFU.

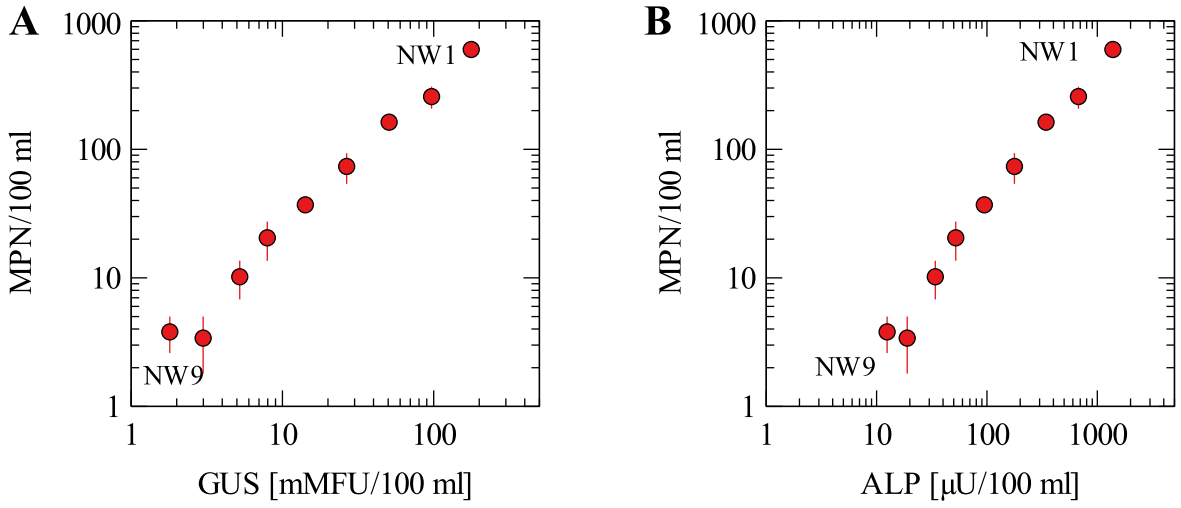

FIGURE 3: MPN (*E. COLI*) PLOTTED AGAINST COLIMINDER® RESULTS. A: GUS; B: ALP. ERROR BARS REPRESENT THE STANDARD DEVIATION OF THREE DETERMINATIONS.

TABLE 4: SUMMARY: ENZYMATIC ACTIVITY OF B-GLUCURONIDASE (GUS) AND ALKALINE PHOSPHATASE (ALP) AND MOST PROBABLE NUMBER (*E. COLI*) OF SERIAL DILUTIONS OF A NATURAL SURFACE WATER SAMPLE. VALUES REPRESENT THE MEAN AND STANDARD DEVIATION OF THREE DETERMINATIONS.

	Dil. fact.	GUS [mMFU/100 ml]		ALP [µU/100 ml]		MPN/100 ml	
		mean	SD	mean	SD	mean	SD
NW1	1	177.03	6.06	1383.03	9.39	598.3	68.1
NW2	2	96.81	0.43	674.48	1.78	257.3	50.1
NW3	4	50.72	0.05	343.07	2.57	162.7	12.7
NW4	8	26.58	0.42	177.40	2.46	73.6	19.7
NW5	16	14.17	0.21	94.59	1.20	37.0	2.5
NW6	32	7.94	0.15	52.16	1.66	20.5	6.9
NW7	64	5.22	0.16	34.03	1.40	10.2	3.4
NW8	128	2.99	0.09	18.94	0.64	3.4	1.6
NW9	256	1.80	0.12	12.45	0.72	3.8	1.2

Evaluation of ColiMinder measurements of a fecally contaminated drinking water sample against a reference method

In a next experiment, serial dilutions of a drinking water sample with artificial fecal contamination were analyzed (FC1-FC6). All three analyses (GUS, ALP and Colilert) yielded a decent linear dependency on the dilution factor (Fig. 6). Like with a natural surface water sample, the sensitivity with GUS and ALP measurements is substantially higher than with a laboratory strain of *E. coli*. The observed range of

~350-10 MPN *E. coli*/100 ml could be covered by both GUS and ALP assays. There is a linear correlation between ColiMinder results and MPN/100 ml for both assays (Fig. 7).

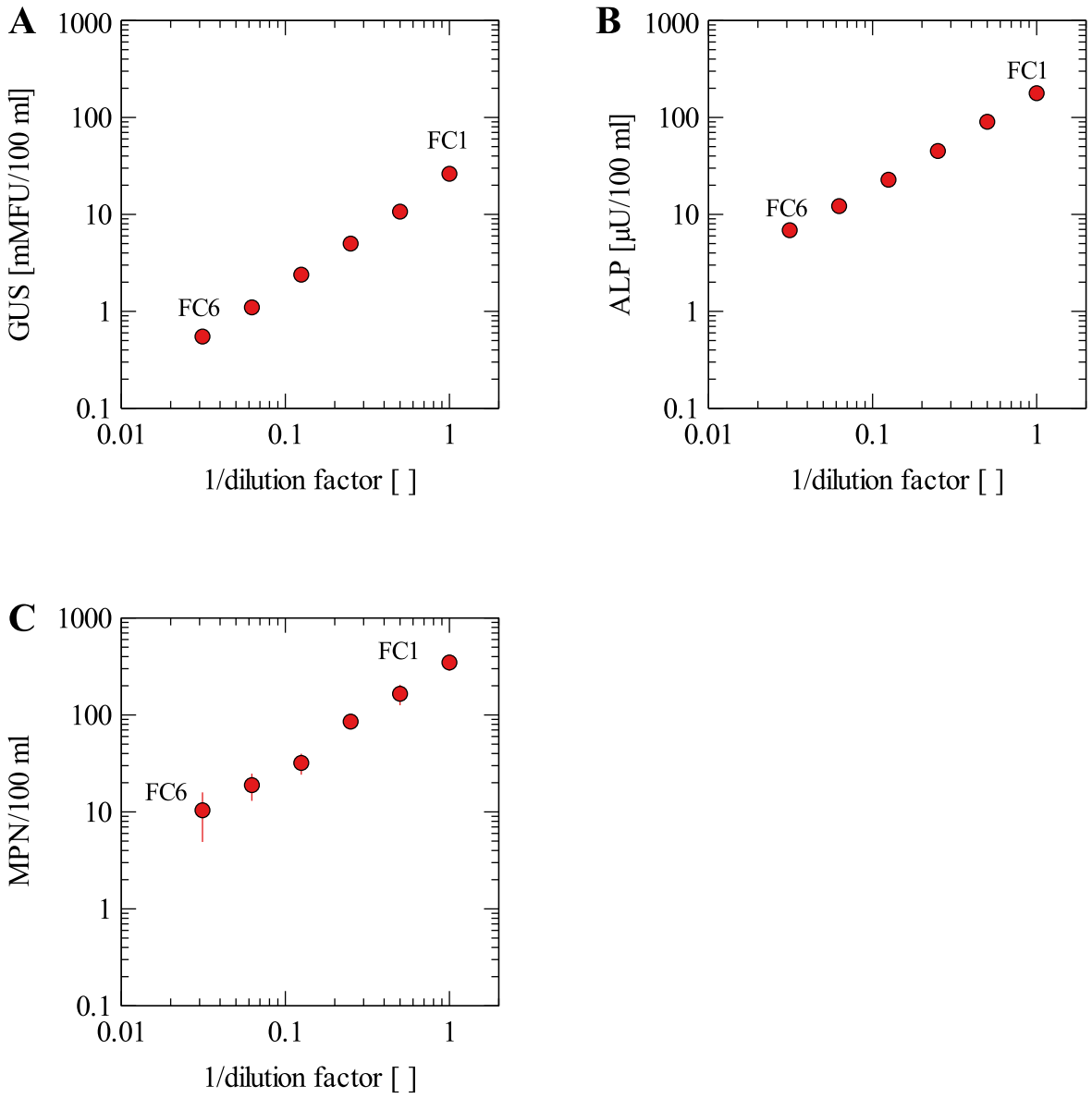

FIGURE 4: ANALYSIS OF SERIAL DILUTIONS OF A DRINKING WATER SAMPLE WITH FECAL CONTAMINATION. A: B-GLUCURONIDASE ACTIVITY (GUS); B: ALKALINE PHOSPHATASE ACTIVITY (ALP); C: IDEXX COLILERT MOST PROBABLE NUMBER (*E. COLI*). THE RESPECTIVE RESULTS ARE PLOTTED AGAINST THE INVERSE OF THE DILUTION FACTOR ON A DOUBLE LOGARITHMIC SCALE. ERROR BARS REPRESENT THE STANDARD DEVIATION OF THREE DETERMINATIONS.

The detection limit for β-glucuronidase is at approx. 10 MPN/100 ml. The reading for pure tap water without additional contamination is not significantly different from a blank with sterile water (Table 6). The ratio of *E. coli* MPN and β-glucuronidase activity in this case is ~ 13 MPN/mMFU.

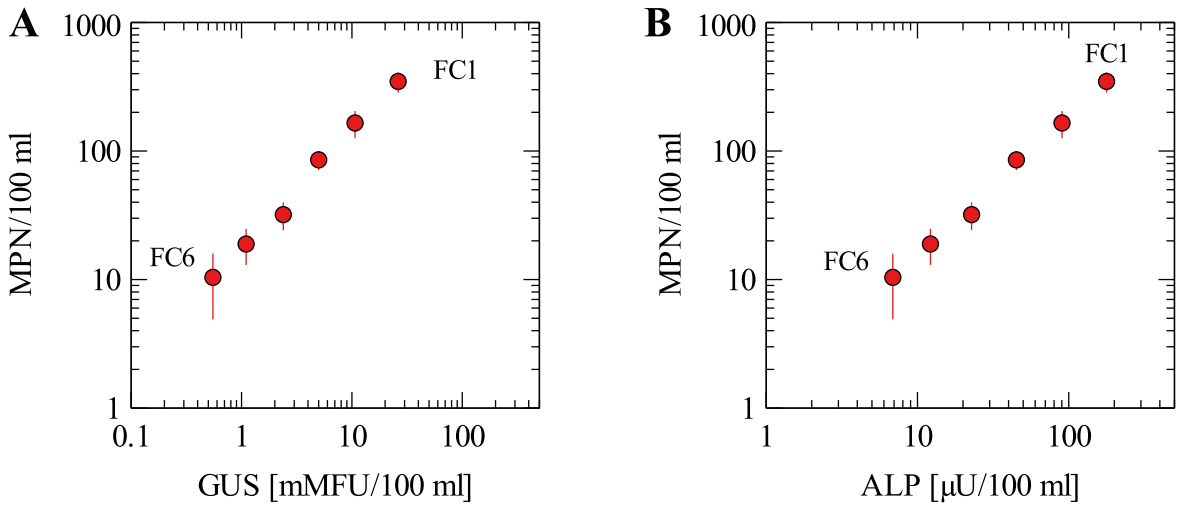

FIGURE 5: MPN (*E. COLI*) PLOTTED AGAINST COLIMINDER® RESULTS FROM A FECALLY CONTAMINATED DRINKING WATER SAMPLE. A: GUS; B: ALP. ERROR BARS REPRESENT THE STANDARD DEVIATION OF THREE DETERMINATIONS.

TABLE 5: SUMMARY: ENZYMATIC ACTIVITY OF B-GLUCURONIDASE (GUS) AND ALKALINE PHOSPHATASE (ALP) AND MOST PROBABLE NUMBER (*E. COLI*) OF SERIAL DILUTIONS OF A DRINKING WATER (TAP WATER) WITH FECAL CONTAMINATION. TAP = PURE TAP WATER WITHOUT ARTIFICIAL CONTAMINATION. VALUES REPRESENT THE MEAN AND STANDARD DEVIATION OF THREE DETERMINATIONS.

	Dil. fact.	GUS [mMFU/100 ml]		ALP [µU/100 ml]		MPN/100 ml	
		mean	SD	mean	SD	mean	SD
FC1	1	26.24	0.83	177.85	2.12	347.7	62.7
FC2	2	10.68	0.09	90.25	1.76	165.3	39.4
FC3	4	5.00	0.22	45.07	0.34	85.4	14.4
FC4	8	2.39	0.16	22.78	0.19	32.0	7.8
FC5	16	1.10	0.07	12.18	0.20	18.9	5.9
FC6	32	0.55	0.08	6.87	0.14	10.4	5.5
Tap	-	0.14	0.05	1.36	0.28	0.0	0.0

The examples of a natural surface water sample and an evidently fecal-contaminated drinking water sample demonstrate that the sensitivity with respect to the detection of *E. coli* with a rapid method is substantially higher under circumstances which are closer to real-life conditions. The comparison to the traditional cultivation-based method reveals that detection limits of both approaches are in a similar range, with the difference, that ColiMinder can provide a result in 15 minutes.

Materials and Methods

Preparation and measurements of natural water sample serial dilutions. Samples were taken from a small local stream ('Weidenbach') and filtered through a paper filter (grade 595 ½) to remove debris. The Weidenbach stream is mainly fed by the discharge of various wastewater treatment plants and thus exhibits highly variable loads of fecal contaminants. Samples were taken approx. 4,700 m downstream of the nearest sewage plant. A portion of the sample was autoclaved, filtered again to remove precipitated carbonates, and kept at +4°C. Serial 1:2 dilutions (NW2-NW9) of the initial sample (NW1) were prepared with cold sterile stream water, stored at +4°C and analyzed with ColiMinder

(GUS and ALP) and IDEXX Colilert (NW1-NW3 1:10; NW4-NW9 undiluted). Blanks were determined with sterile stream water.

Preparation and measurements of fecally contaminated drinking water. Drinking water from a tap in Zwerndorf, Austria, was artificially contaminated by adding 20 ml/l of water from rinsing a toilet brush. The resulting sample (FC1) was serially diluted 1:2 with cold (+4°C) tap water (FC2-FC6). Samples were kept at +4°C during measurements and dilutions were readily prepared before analysis with ColiMinder (GUS and ALP). In parallel, MPN/100 ml was determined with IDEXX Colilert (FC1-FC2 1:10; FC3-FC6 undiluted). Blank measurements with ColiMinder were performed with sterile-filtered tap water (0.45 µm PES syringe filter).

10 Time to Result

The time to result for a single determination in ColiMinder is 15 minutes and independent of the level of contamination or sample matrix. Consequently, a measurement every 15 minutes is possible, but including the recommended automatic cleaning procedure, a determination every 30 minutes is feasible.

11 Material used

I. **Hardware:** ColiMinder device
II. **Reagents:**
β-glucuronidase (*E. coli*)***:***

CM.GUS Quick Detect Reagent A (prepared from Reagent A-1 and Reagent A-2)
CM.GUS Quick Detect Reagent B
CM Quick Clean Wash Solution Concentrate
Alkaline phosphatase (Total Activity)**:**
CM.ALP Quick Detect Reagent A
CM.ALP Quick Detect Reagent B
CM Quick Clean Wash Solution Concentrate
Additionally, deionized rinsing water is required.

12 Mass production capacity

I. **ColiMinder devices:** The instrument is entirely made in Austria. Current production capacities are approx. 50 devices per year. This can be extended according to higher demand.
II. **Reagents:** All reagent assays are entirely manufactured by VWMS in Austria. Current capacity is approx. 3,000 reagent kits á 1,000 measurements per year and can be amended to higher demand immediately.

THE COLIMINDER INSTALLED FOR MONITORING DRINKING WATER BOTTLING IN SOUTH AFRICA

13 Testing methodology

Operation and maintenance of ColiMinder® and interpretation of data can be performed by trained personnel and do not require specific knowledge of scientific or technical experts. Training is provided by VWMS experts online or on site, taking approx. ½ day for staff with low technical expertise.

The minimum steps for a single measurement are as follows:

- Capturing the sample and immersing ColiMinder's sample line tubing into the sample container.
- Starting the measurement via the User Interface on a provided Tablet PC or any smartphone connected to ColiMinder. The measurement will be carried out by the device, and the result is displayed.
- Data interpretation. All results are provided online and illustrated in various graphical representations. Online data provision enables interpretation of results by experienced data experts remotely. Any data can be exported in common data- and image formats.

Occasional additional steps include the exchange of consumables (switching reagent bottles), refills of rinsing water, emptying of waste containers, and maintenance tasks:

- Start-up after >1 day since the last measurement (a few clicks in the User Interface, approx. 15 minutes duration of the procedure)
    1. Initiate a flush of the reagent lines (1 minute)
    2. Trigger a cleaning cycle via the User Interface (15 minutes)
- Exchange of reagents after 250, 500, or 1,000 measurements (5 minutes):
    1. Open the housing, unscrew empty reagent containers
    2. Unpack and prepare new reagents and wash solution according to the User Manual
    3. Reattach fresh reagent containers, close housing

4. Initiate a flush of the reagent lines via the User Interface

- Refill of rinsing water (5 minutes)
    1. Refill the rinsing water container with clean deionized water
- Emptying the waste container if used (5 minutes)
- Battery exchange – in case recharging is not possible (ERU and Mobile, 5 minutes)
- Preparing the device for long-term storage or shipping according to the User Manual (30 minutes)
- Maintenance: Exchange of internal tubing and fittings every other year (1 hour)

Annex A: List of peer-reviewed publications

Margot Cazals, Rebecca Stott, Carole Fleury, François Proulx, Michele Prevost, Pierre Servais, Sarah Dorner, Jean-Baptiste Burnet; (February 2020) Near-real time notification of water quality impairments in recreational freshwaters using rapid online detection of β-D-glucuronidase activity as a surrogate for *Escherichia coli* monitoring; February 2020 - Science of The Total Environment

Emile Sylvestre, Jean-Baptiste Burnet, Patrick Smeets, Gertjan Medema, Michele Prevost, Sarah Dorner (December 2019) Can routine monitoring of *E. coli* fully account for peak event concentrations at drinking water intakes in agricultural and urban rivers? December 2019 Water Research 170:115369

Jean-Baptiste Burnet, Emile Sylvestre, Jonathan Jalbert, Sandra Imbeault, Pierre Servais, Michele Prevost, Sarah Dorner, (2019), Tracking the contribution of multiple raw and treated wastewater discharges at an urban drinking water supply using near real-time monitoring of b-D-glucuronidase activity, Water Research 164 (2019)

Jean-Baptiste Burnet, Quoc Tuc Dinh, Sandra Imbeault, Pierre Servais, Sarah Dorner, Michèle Prevost - Autonomous online measurement of ß-D-glucuronidase activity in surface water: is it suitable for rapid *E. coli* monitoring? Water Research 152 (2019) 241-250

Philipp Stadler, Luke C. Loken, John T. Crawford, Paul J. Schramm, Kirsti Sorsa, Catherine Kuhn, Domenico Savio a,h,i, Robert G. Striegl, David Butman, Emily H. Stanley, Andreas H. Farnleitner, Matthias Zessner - Spatial patterns of enzymatic activity in large water bodies: Ship-borne measurements of beta-D-glucuronidase activity as a rapid indicator of microbial water quality; Science of the Total Environment 651 (2019) 1742–1752

Philipp Stadler, Günter Blöschl, Wolfgang Vogl, Juri Koschelnik, Markus Epp, Maximilian Lackner, Markus Oismüller, Monika Kumpan, Lukas Nemeth, Peter Strauss, Regina Sommer, Gabriela Ryzinska-Paier, Andreas H. Farnleitner and Matthias Zessner - Real-time monitoring of beta-D-glucuronidase activity in sediment laden streams: A comparison of prototypes. Water Research, Volume 101, 15 September 2016, Pages 252-261 (2016, May). Paper

Demeter K, Burnet J-B, Stadler P, Kirschner A, Zessner M, Farnleitner AH, Automated online monitoring of fecal pollution in water by enzymatic methods, Current Opinion in Environmental Science & Health, https://doi.org/10.1016/j.coesh.2020.03.002.

Annex B: Further references

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Annex C: Abstract of Customer References

Drinking Water and Food & Beverage

1. Latest customer: Romaqua, Romania, Europe – the ColiMinder monitoring microbiological quality of wells for still and naturally carbonated mineral water
2. WSD – Water Supply Department, Hong Kong
3. De Watergroep, Belgium
4. Bathurst Council, Australia
5. Mekorot, Israel
6. Seoul Water Institute at the Seoul Metropolitan Government, Korea
7. EVN, Austria
8. Gemeinde Weiden an der March, municipal drinking water supply, Austria (home base municipality)
9. Agrana – fruit production and washing lines, Austria

Surface / Bathing / Raw Water

1. Latest customer: SERAMM / SUEZ – monitoring bathing water at Marseille beaches, France
2. Eau de Paris – monitoring Seine river, other bathing waters and raw water intake for drinking water production
3. NIWA, New Zealand
4. KIT – Karlsruhe Institute of Technology, monitoring karst springs and waters, all over Europe and in Vietnam
5. Polytechnique de Montréal – 6 ColiMinder devices for scientific evaluation of the technology, several studies published
6. AgResearch, New Zealand

Wastewater

EXAMPLES OF COLIMINDER INSTALLATIONS: (FROM LEFT TO RIGHT): BATHING WATER (PARIS), RAW WATER FOR DRINKING WATER (AUSTRALIA), MUNICIPAL DRINKING WATER (AUSTRIA), NATIONAL DRINKING WATER NETWORK (BELGIUM)

1. Latest customer: MSD Cincinnati in cooperation with USEPA, using 2 ColiMinder devices for monitoring process performance in wastewater treatment, USA
2. DSD – Drainage Services Department, monitoring wastewater effluent in one of world's biggest WWTP, Hong Kong
3. Trojan UV in cooperation with Western University, Ontario, Canada

Membrane Integrity

1. Since 2016: DHI Group, Denmark, monitoring membrane integrity in the effluent of a hospital's MBR plant discharging into a recreational water body. Awarded by the Danish EPA as "Best Available Technology".

Process / Industrial Water

1. Since 2017: company not mentionable due to NDA, monitoring of microbiological contamination in metal working fluids in industrial production process, Europe