ColiMinder rapid microbiology by VWM SOLUTIONS

ALP Total Activity Measurements

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This document contains results of systematic tests to provide information on reproducibility, linearity, limit of detection of the ColiMinder measuring Total Microbiological Activity.

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ColiMinder signal response

ColiMinder signal response vs. concentration of alkaline phosphatase (ALP). Standard solutions with known concentrations of ALP were prepared covering a broad dynamic range from 0 – 10,000 µU/100 ml and measured with ColiMinder (triple determinations). The data was plotted against the nominal concentration of the standards and fitted by linear regression (y = ax + b). Due to the heteroscedasticity of the data, a weighted fit was used. The applied weights were calculated according to:

$$w_i = \frac{s_i^{-2}}{\sum_i s_i^{-2}/n}$$

w_i = weight for data point *i*
s_i = standard deviation of data point *i*
n = number of data points

The data show a linear response in the range of 0 – 2,000 µU/100 ml, and a deviation from linearity above 2,000 µU/100 ml, as indicated the trend in increasing positive residuals in this range (Table 1, Figs. 1 and 2).

Table 1: Parameters of linear regressions for two different ALP concentration ranges.

	0 – 10,000 µU/100 ml	0 – 2,000 µU/100 ml
Slope (a)	1.08 ± 0.01	1.08 ± 0.01
Intercept (b)	3.30 ± 0.45	3.33 ± 0.52
Coefficient of determination (R^2)	1.0	0.9999
Root mean square error (RMSE)	1.7482	1.7440

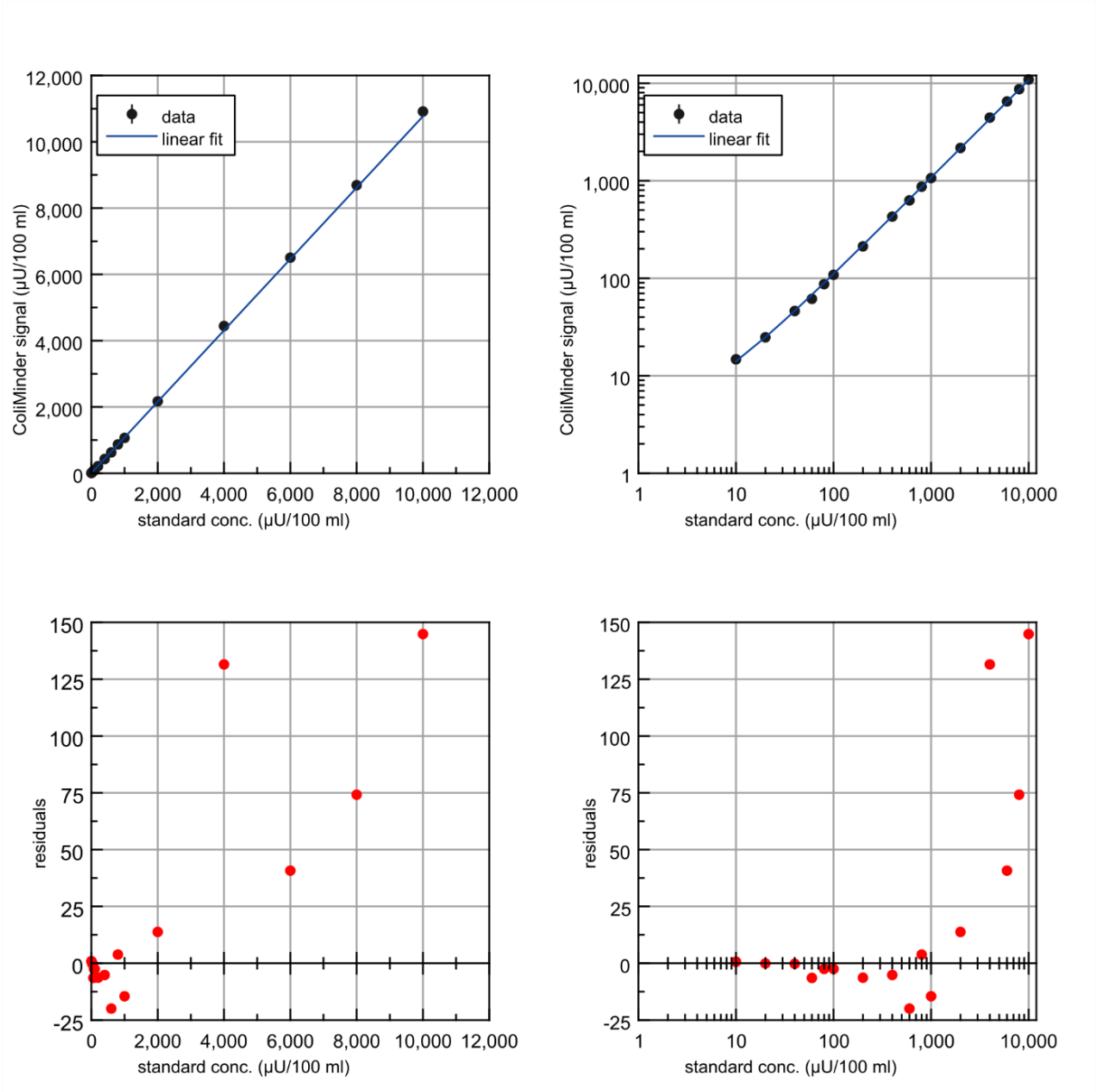

Figure 1: Plots of ColiMinder signal response vs. concentration and residuals. Top: Signal response of ColiMinder in a range of 0 – 10,000 µU/100 ml on a linear scale (left) and double log-scale (right). Bottom: residual plots corresponding to the linear fits in the respective top panels.

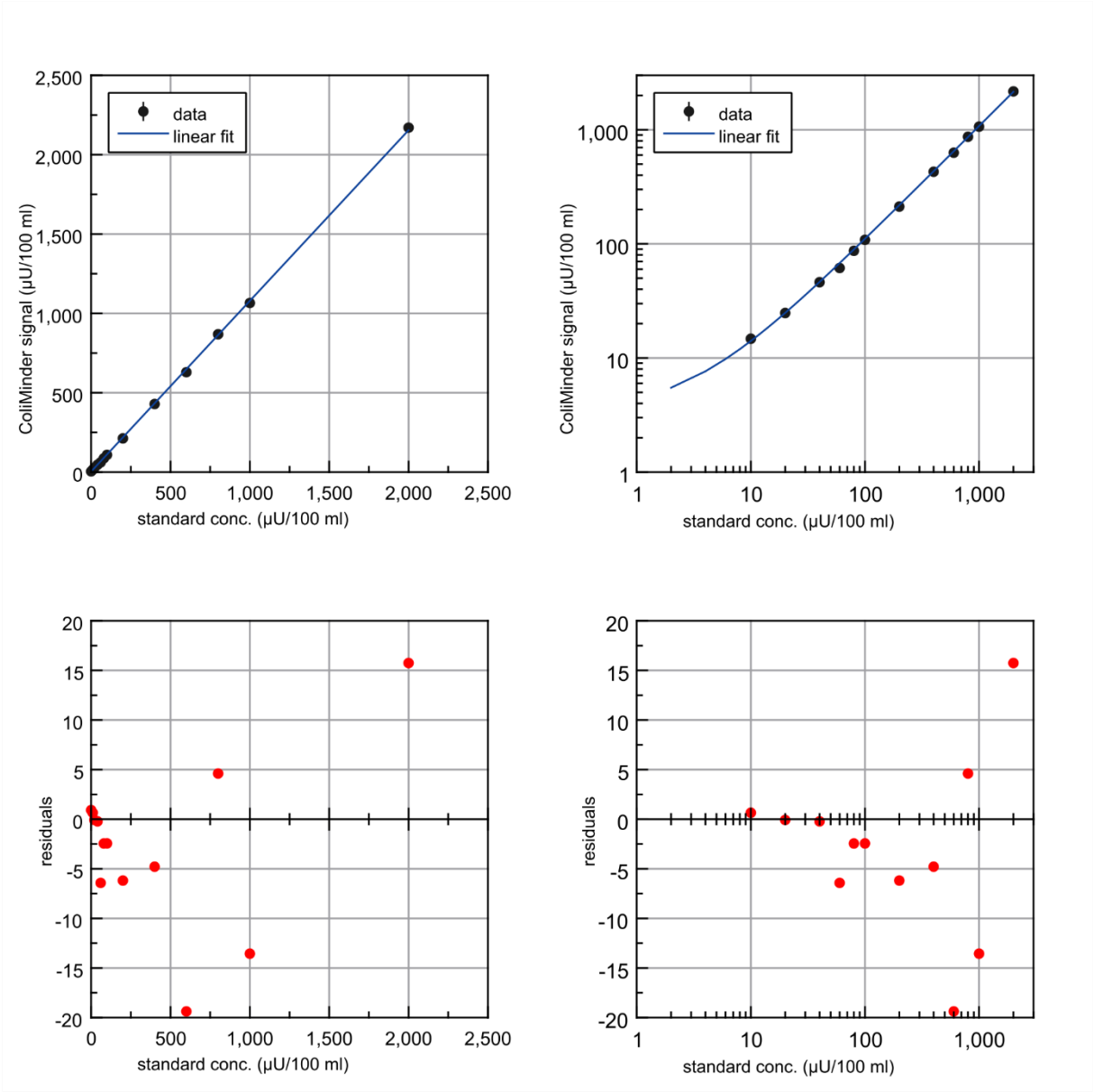

Figure 2: Plots of ColiMinder signal response vs. concentration and residuals. Top: Signal response of ColiMinder in a range of 0 – 2,000 µU/100 ml on a linear scale (left) and double log-scale (right). Bottom: residual plots corresponding to the linear fits in the respective top panels.

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) are valid for samples in clear water without dissolved organic compounds, buffering agents or high contents of inorganic substances. Values are shown in Table 2.

Table 2: Detection limits for ALP measurements. *Limits, if the result of a blank measurement of sterile distilled/deionized water is subtracted from the measurement result.

Limit of detection (LOD)	4.68 µU/100 ml 0.36 µU/100 ml *
Limit of quantification (LOQ)	5.27 µU/100 ml 1.20 µU/100 ml *

Remarks:

Limit of detection (LOD)

The LOD was determined as the mean of *m* blank results plus 3 × the estimated standard deviation of *m* single blank results divided by the square root of the number of replicate observations per single measurement (*n*). *m* = 15.

$$LOD = y_b + 3 * \frac{s_0}{\sqrt{n}}$$

If a *dd*H_2O blank measurement result is subtracted from the measurement results, then

$$LOD = 3 * s_0 * \sqrt{\frac{1}{n} + \frac{1}{n_b}}$$

s_0 = standard deviation of *m* blank measurement results
y_b = average of *m* blank measurement results
n = 1 (number of determinations averaged to be reported as a single result)
n_b = 1 (number of blank determinations averaged to be subtracted from a single result)

The factor of 3 is very common in analytical chemistry and results in an error probability for Type I or Type II errors of 7% when the results are normally distributed, e.g., if the average of several measurement results of the same sample is equal to the LOD, there is a 7% probability that it is in fact a blank, and not a sample with a low concentration (Type I error).

Limit of quantification (LOQ)

The LOQ was determined according to LOD, using the usual factor of 10.

$$LOQ = y_b + 10 * \frac{s_0}{\sqrt{n}}$$, or

$$LOQ = 10 * s_0 * \sqrt{\frac{1}{n} + \frac{1}{n_b}}$$

Example: ColiMinder vs. cultivation-based methods – 3M Petrifilm Aqua Heterotrophic Count Plates (AQHC)

Heterotrophs are organisms which require an organic carbon source. All animals, fungi, and most bacteria are heterotrophs. The traditional method to detect heterotrophs is to cultivate them on a suitable growth medium providing a carbon source and count the resulting colonies (heterotrophic count plates).

ColiMinder evaluates a different parameter by directly measuring the activity of Alkaline Phosphatase (ALP) in a sample. Phosphatases (Enzyme class EC 3.1.3) are essential enzymes involved in many cellular processes in almost all organisms. One of the many families of phosphatases is the family of Alkaline Phosphatases (EC 3.1.3.1), which is especially abundant in bacteria, fungi, and animals. Thus, the set of heterotrophs and the set of organisms having ALPs overlap to a high degree. The underlying principle of ColiMinder measurements is that the amount of ALP in a sample, determined via ALP activity, correlates with the number of organisms in the sample.

The following comparison shows, that results of both methods (number of colonies of heterotrophs on a medium per volume of sample, and ALP activity per volume of sample), although highly different in principle, provide valid and consistent metrics for the abundance of organisms in a water sample.

Materials and Methods

Still mineral water from a local brand was sterile filtered (0.45 µm cellulose acetate filter) and spiked with contaminated surface water from a local stream to yield a final ALP concentration of approx. 100 µU/100 ml with ColiMinder. Six serial 1:2 dilutions were prepared in sterile mineral water and analyses were done immediately after sample preparation. Three consecutive measurements of each sample were done with ColiMinder. For 3M Petrifilm AQHC analyses, the samples were further serially diluted 1:10, if necessary, in sterile mineral water and 1 ml of sample was spotted on a film (three determinations each). Films were incubated at 36°C for 44 ± 4 h, and plates with counts between ten and 250 colonies were considered for analysis.

Results and discussion

Results with ColiMinder show a linear progression with dilution, while plate counts deviate from linearity at the highest contaminated sample (Fig. 3). The variation between repeated determinations is lower with ColiMinder (intra-assay coefficient of variation: ColiMinder 0.89%, AQHC counts 11.14%). The average relation between ALP activity and plate counts is approx. 2 CFU/ml per µU/100 ml.

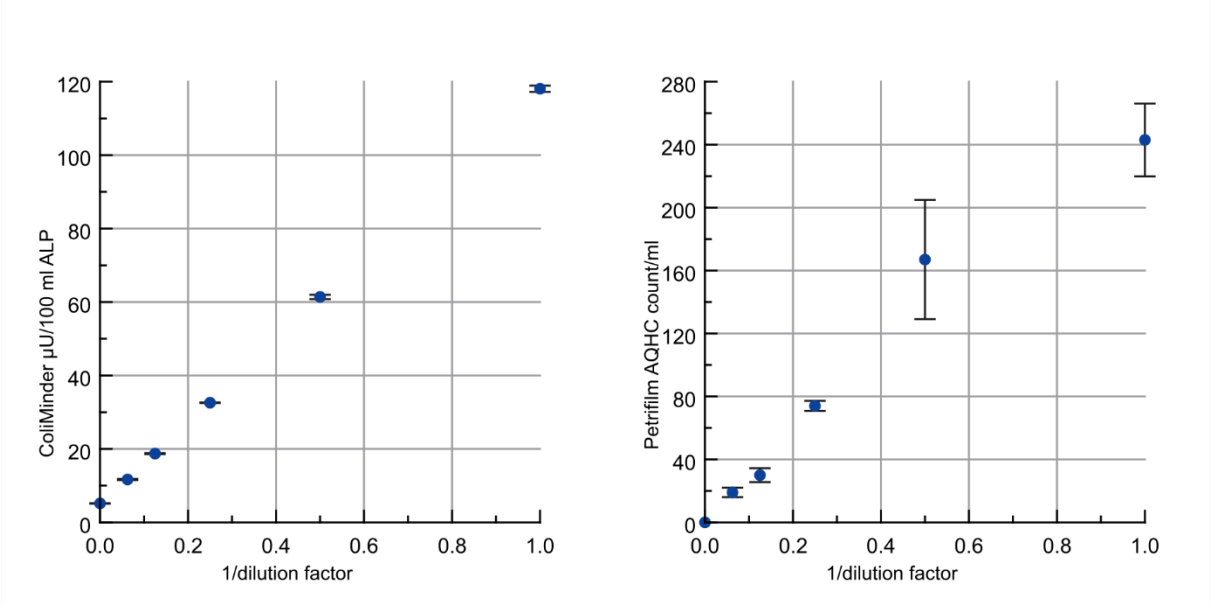

Figure 3: ColiMinder readings (left) and 3M Petrifilm AQHC counts (right) against the inverse of the dilution factor. Error bars represent the standard deviation of three determinations.

In general, there is a good correlation between 3M Petrifilm AQHC counts and ColiMinder readings (Pearson's *r* = 0.98, Fig. 4).

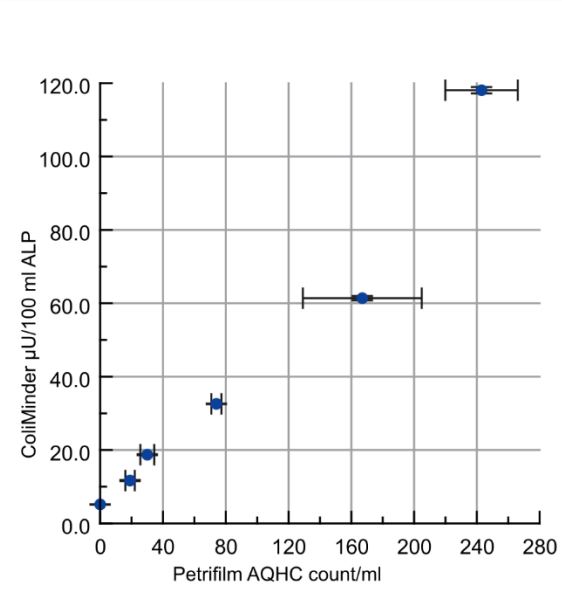

Figure 4: Correlation between ColiMinder ALP µU/100 ml and 3M Petrifilm AQHC counts/ml. Error bars represent the standard deviation of three determinations.

It is important to note that due to the complete difference of the biological parameter being evaluated (ability to grow on a certain medium vs. level of enzymatic activity), the relation between ALP activity/volume and CFU/volume can be different depending on the type of sample and the particular biological composition of organisms in a sample. Different species can exhibit different amounts of ALP activity per individual, as well as they can show different growth behavior/culturability in cultivation-based methods. However, the relation is quite constant when the principal sample parameters are constant (e.g., in constant monitoring applications of similar water sources). The relation in the present comparison provides a representative example of bottled mineral water contaminated with organisms from a surface water source.