Illuminating microbial contamination risk: the usability of fluorimetry for rapid groundwater assessment in low-resource contexts

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**Highlights**

- Tryptophan-like fluorescence (TLF) can complement *E. coli* as a risk indicator.
- With reference to *E. coli*, TLF differentiated risk at three levels of concentration [50% FPRs - 20%].
- Fluorimetry suffers less method-induced variability than bacteriological analyses.
- TLF is equally or better suited to WHO and UNICEF indicator criteria than *E. coli*.
- TLF is useful for pre-screening, monitoring and demonstrating risk in groundwater.

**Methods**

**Where:** rural KwaIle County, Kenya.

**What:** shallow unconfined aquifer

**How:** laboratory testing. Calibration standards of 0, 0.5, 1, 2, and 5 ppb were prepared from L-trypophan (Acris Organics, USA) in deionized water.

**Comparision with E. coli Risk Class**

<table>
<thead>
<tr>
<th>E. coli per 100 mL</th>
<th>Risk Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>low</td>
</tr>
<tr>
<td>1 - 10</td>
<td>intermediate</td>
</tr>
<tr>
<td>11 - 100</td>
<td>high</td>
</tr>
<tr>
<td>&gt;100</td>
<td>very high</td>
</tr>
</tbody>
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**E. coli** detection methods are relatively slow, complicated, and expensive - there is demand for something better (UNICEF’s rapid *E. coli* detection Target Product Profile for example). We propose that risk assessments could be improved by a complementary indicator, tryptophan-like fluorescence (TLF). The TLF peak (excitation/emission at 275/340 nm) reflects concentrations of compounds that have similar fluorescence characteristics as the amino acid, tryptophan. It is associated with microbial breakdown of labile organic carbon. Facially contaminated water has intense TLF peaks.

**Comparison with E. coli Risk Class**

The boxes show medians and span lower to upper quartiles, the whiskers show the lowest and highest data within 1.5 times the interquartile range. The horizontal dotted lines on the first chart show TLF = 0.95 ppb and TLF = 3.67 ppb: these thresholds divide the TLF results into three levels: low, medium and high. False negative and/or positive error rates were around 20% for each level. Using *E. coli* as a reference, TLF does not distinguish intermediate risk from baseline conditions; however, *E. coli* is an imperfect indicator and the utility of TLF should not be ignored to it.

**Method-Induced Variability**

The fluorimetry results were more precise than the bacteriological results. The TLF duplicates showed lower relative percent difference (RPD) and better agreement between pairs than the duplicates or replicates of any of the bacteriological methods. Agreement here is defined as the proportion of pairs that indicate the same risk class. For the TLF data this was based on the three groupings defined above.

**Suggested Applications**

1) Pre-screening: TLF enables larger samples sizes and can provide information quickly enough to inform priorities. WP's with high TLF could be considered for a priority and then followed up with *E. coli* sampling. WP's with low TLF may warrant further investigation, especially when coupled with high sanitary inspection scores. Instead of sampling many WP's once, a selection could be tested in the appropriate conditions, peak-picking fluorimetry is well matched to 75% of the criteria in UNICEF's rapid risk assessment Target Product Profile (August 2017).

**Challenges and Limitations**

1) Calibration: for TLF results to be comparable between studies, we need a standardized protocol for calibration to account for probe sensitivity.

2) Interpretation: the relationship between TLF and *E. coli* will be context specific and baseline TLF conditions will vary. Interpretation of TLF results must be with reference to a baseline range.

3) Interference: TLF signal strength is impacted by hemic content, temperature, turbidity and pH. TLF fluorimetry is best applied in low-humic groundwaters with consistent temperature, low turbidity (<50 NTU) and pH between 5 and 8.

Other substances that fluoresce in the TLF range include polyyclic hydrocarbons, pharmaceutically active compounds, and pollutants from plastic, petroleum, paper, leather and textile processing.

In this study, sample turbidity was low (<70 FNU, 95% < 1 FNU, 95% < 10 FNU and none exceeded 50 FNU) and pH was circumneutral (mean 7.1; SD 0.2).

Sample temperature range was 28 to 32 °C and lab work showed negligible impact of temperature change in that range.

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2) Regulatory or surveillance monitoring: Precision and low marginal cost of sampling make TLF fluorimetry well-suited for capturing changes in risk. Larger-scale spatiotemporal trends are less likely to be obscured by method-induced variability or short-term water quality fluctuations.

3) Real-time demonstrations for communicating with stakeholders: Different water sources can be compared and changes in water quality can be captured and shown in real time. To encourage handwashing and safe storage, it is possible to show the effect of putting hands into clean water.

**Funding**

This research was supported by the British Natural Environment Research Council (NERC), Economic and Social Research Council (ESRC) and Department for International Development (DFID) through the Unlocking the Potential of Groundwater for the Poor Consortium Grant (NE/M008894/1). Data will be publicly available from the National Geoscience Data Centre and the UK Data Archive.

https://upgro.org/consortium/grf-for-good/