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 [FNRs/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.

Introduction

Low-cost, practical in-field methods are necessary if water quality information is to be available in support of decision making in low resource settings. Drinking water microbial contamination risk is typically assessed with a risk indicator approach that relies on *Escherichia coli.*

***E. coli* per 100 mL**	**Risk Class**
<1	low
1 – 10	intermediate
11 – 100	high
>100	very high

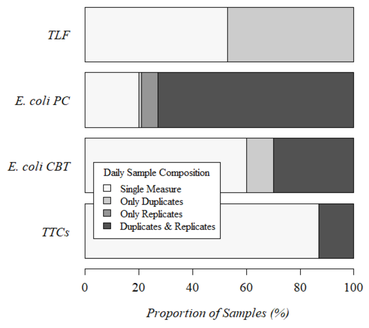
E. coli detection methods are relatively slow, complicated, and expensive – there is demand for something better (UNICEF's rapid *E. coli* detection Target Product Profile for example). We propose that risk assessments could be improved by a complementary indicator, tryptophan-like fluorescence (TLF). 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. Faecally contaminated water has intense TLF peaks.

Methods

Where: rural Kwale County, Kenya,
What: shallow unconfined aquifer
37 water points (WPs):
- 12 open wells (OWs)
- 14 covered wells with handpumps (CHs)
- 11 boreholes with handpumps (BHs)

When: June 2016 (end of the long rainy season)
- 8 WPs daily for 3 weeks

March 2017 (intensified dry season)
- 5 WPs daily for 2 weeks
- 29 WPs visited once each



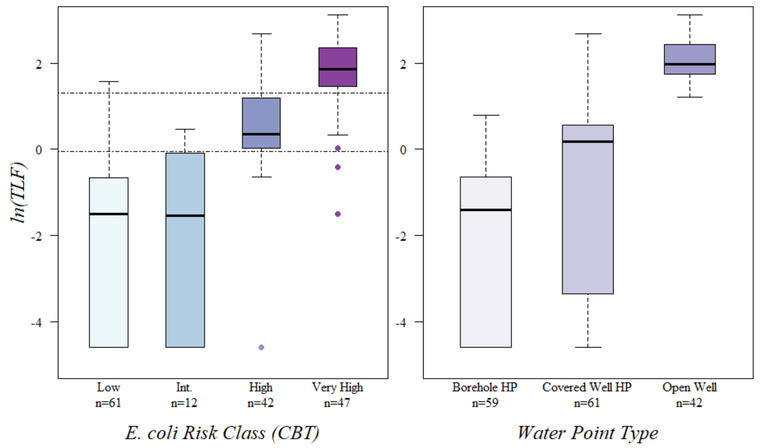
How:

Method	Indicator	Manufacturer	Daily Samples
CBTs	*E. coli*	Aquagenx, North Carolina, USA	242
PC (m-ColiBlue24)	*E. coli*	Hach, Colorado, USA	70
PC (laurel sulphate)	TTCs	DelAgua, Surrey, UK	161
In-situ fluorimetry	TLF	CTG, Surrey, UK	162

TLF was measured with three commercially available UviLux probes, LED UV-based portable fluorimeters that target the 280±30/360±50 nm excitation/emission peak. TLF readings were recorded for 3 minutes (manually in 2016 and both manually and using a logger in 2017). Median values were selected and 2017 data showed near-perfect agreement between manually and automatically recorded results (Pearson's r = 0.9996, paired t-Test p < 0.001).
The raw TLF data was corrected for probe sensitivity using calibration curves generated through laboratory testing. Calibration standards of 0, 0.5, 1, 2, and 5 ppb were prepared from L-tryptophan (Acros Organics, USA) in deionized water.

Comparison with *E. coli* Risk Class

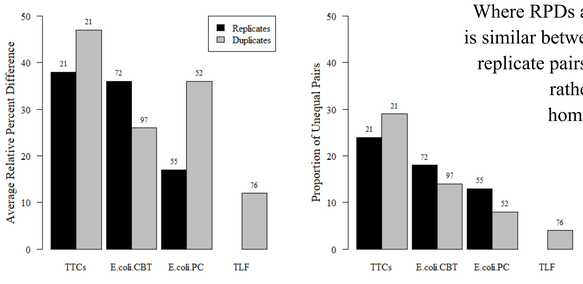
There were significant monotonic relationships for all two-way comparisons of the fluorimetry and bacteriological results ($p < 0.001$). However, all relationships had substantial scatter, with Kendall's τ tie-corrected rank correlation coefficients ranging from 0.57 (TLF and CBT *E. coli*) to 0.77 (the two *E. coli* tests). Since microbial water quality sampling is ultimately concerned with assessing risk, further analyses grouped the bacteriological data by risk classes.



The boxes show medians and span lower to upper quartiles, the whiskers show the lowest and highest datums 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 anchored to it.

Method-Induced Variability

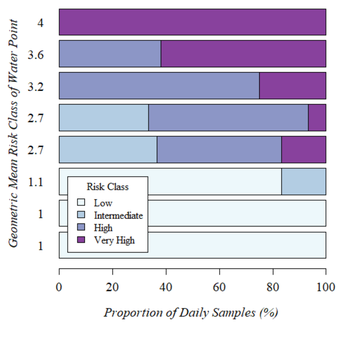
The fluorimetry results were more precise than the bacteriological results. The TLF duplicates showed lower relative percent difference (RPD) and better agreement between pairs than the duplicates or replicates of any of the bacteriological methods. Agreement here is defined as the proportion of pairs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



Where RPDs and level of agreement is similar between duplicate pairs and replicate pairs, analytical variability rather than source material homogeneity is controlling sample precision.

The speed and in-situ nature of fluorimetry reduces analytical variability. The average and median standard deviations of auto-logged TLF measurements (n = 76) were 0.04 and 0.03 ppb. Precision was best at low and high concentrations.

Change in precision with concentration may also be expected for CBT results. When the average risk class of a WP was between 'intermediate' and 'high', the daily samples showed substantial variability, spanning three risk classes. Various conclusions may have been drawn if these WPs were sampled once.



Criteria Comparison

Ideal Indicator Criteria (WHO 2011)	***E. coli***	**TLF**
universally present in faeces at higher concentrations than pathogens	✓	✓
persist and respond to treatment in a similar manner to pathogens	! viruses and protozoa	✓ size, resilience, context*
not be pathogenic	! some pathogenic strains	✓ general characteristic
be simply and inexpensively detected	! time, consumables, facilities	✓ in-situ, no consumables
not multiply in natural waters	! context dependent	! interpret against baseline

*TLF is linked to labile carbon content, a potential advantage for indicting risk from *Legionella*, *Vibrio cholerae*, *Naegleria fowleri*, and *Acanthamoeba*

Used 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).

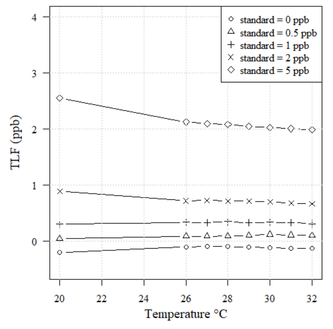
Challenges and Limitations

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 humic content, temperature, turbidity and pH. Peak-picking 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 polycyclic hydrocarbons, pharmaceutically active compounds, and pollutants from plastic, petrochemical, paper, leather and textile processing.

In this study, sample turbidity was low (70% < 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° and lab work showed negligible impact of temperature change in that range.



Suggested Applications

1) **Pre-screening:** TLF enables larger samples sizes and can provide information quickly enough to inform priorities. WPs with high TLF could be considered high risk and not a priority for *E. coli* sampling. WPs with low TLF may warrant further investigation, especially when coupled with high sanitary inspection scores. Instead of sampling many WPs once, a selection could be tested with duplicate/replicate sampling — enabling better risk estimation with geometric means.

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.

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