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# Tryptophan-like fluorescence as a measure of microbial contamination risk in groundwater

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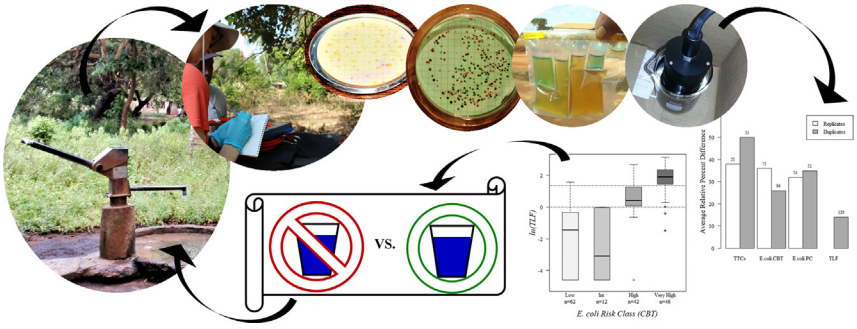
## HIGHLIGHTS

- Tryptophan-like fluorescence (TLF) can complement *E. coli* as a risk indicator.
- Both TLF and *E. coli* distinguish low/intermediate, high and very high risk sources.
- TLF has negligible method-induced variability, unlike bacteriological analyses.
- TLF is useful for pre-screening, monitoring and demonstrating risk in groundwater.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Microbial water quality is frequently assessed with a risk indicator approach that relies on *Escherichia coli*. Relying exclusively on *E. coli* is limiting, particularly in low-resource settings, and we argue that risk assessments could be improved by a complementary parameter, tryptophan-like fluorescence (TLF). Over two campaigns (June 2016 and March 2017) we sampled 37 water points in rural Kwale County, Kenya for TLF, *E. coli* and thermotolerant coliforms (total n = 1082). Using three World Health Organization defined classes (very high, high, and low/intermediate), risk indicated by TLF was not significantly different from risk indicated by *E. coli* ($p = 0.85$). However, the TLF and *E. coli* risk classifications did show disagreement, with TLF indicating higher risk for 14% of samples and lower risk for 13% of samples. Comparisons of duplicate/replicate results demonstrated that precision is higher for TLF (average relative percent difference of duplicates = 14%) compared to culture-based methods (average RPD of duplicates ≥ 26%). Additionally, TLF sampling is more practical because it requires less time and resources. Precision and practicality make TLF well-suited to high-frequency sampling in low resource contexts. Interpretation and interference challenges are minimised when TLF is measured in groundwaters, which typically have low dissolved organic carbon, relatively consistent temperature, negligible turbidity and pH between 5 and 8. TLF cannot be used as a proxy for *E. coli* on an individual sample basis, but it can add value to groundwater risk assessments by improving prioritization of sampling and by increasing understanding of spatiotemporal variability.



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## 1. Introduction

Improving water quality is crucial to the United Nations' Sustainable Development Goal 6.1, ensuring universal access to safely managed drinking water. Globally, 25% of people lack access to water free from microbial contamination (JMP, 2017); in Africa, the estimate doubles to 50% (Bain et al., 2014). The resulting disease burden is difficult to quantify but for low- and middle-income countries, half a million diarrheal deaths recorded in 2012 were attributed to microbially contaminated water (Prüss-Ustün et al., 2014). Beyond mortality, there are persistent physical and cognitive morbidity impacts, especially for children (Guerrant et al., 2002).

Groundwater usually has better microbial quality compared to surface water, but it can be vulnerable to anthropogenic impact. Regional estimates of groundwater microbial contamination range from 78% to 97% of unprotected water points and 10% to 41% of boreholes (Bain et al., 2014). This contamination has large repercussions because direct access to groundwater accounts for a third of global water supply (27.3% protected, 7.4% unprotected), coming second only to piped networks (Bain et al., 2014). Work is ongoing to better understand and manage groundwater contamination and risk assessment is central to that effort (Murphy et al., 2017).

Assessment of microbial contamination is ultimately concerned with the presence of pathogens; however, sampling for pathogens is difficult: there are many types, they frequently occur in low concentrations, and differentiating between infectious and non-viable organisms is challenging (Cangelosi and Meschke, 2014). As a result, an indicator approach using coliform bacteria has been common for the last century. As a common enteric species that is relatively easy to culture, *Escherichia coli* (*E. coli*) is the preferred indicator (WHO, 2011).

*E. coli* are thermotolerant coliforms (TTCs), meaning that they are culturable and ferment lactose at 44 °C. In addition to *Escherichia*, the TTC subgroup also includes three other genera (*Klebsiella*, *Enterobacter* and *Citrobacter*). The ratio of *E. coli* to all TTCs is variable (Garcia-Armisen et al., 2007; Hamilton et al., 2005; WHO, 2012), but it is not uncommon for TTCs to be used as a proxy for *E. coli* (WHO, 2011). For risk assessment purposes, *E. coli* (or TTC) results are often interpreted by way of a four-tier ordinal risk classification scheme based on either most probable number (MPN) or colony forming units (CFU) per 100 ml (WHO, 2011). The risk classes are low (<1), intermediate (1−10), high (11−100), and very high (>100).

Although widely regarded as a successful approach, use of *E. coli* is costly in terms of time requirement and consumables. Furthermore, as explained later in this section, the absence of *E. coli* in drinking water cannot be relied upon as a certain indication of safety. In this study we show how an additional parameter, tryptophan-like fluorescence (TLF), may help address these disadvantages. The TLF peak (centred on excitation/emission at 275/340 nm) is so named because it reflects concentrations of compounds that have similar fluorescence characteristics as the amino acid tryptophan. The constituents that produce TLF are fractionated into three size classes (Baker et al., 2007). The >2.7 μm fraction includes particulate organic matter that cause a detrimental apparent signal through scattering of light. The middle size class (0.2 μm to 2.7 μm) includes bacteria, which contribute directly to TLF. The remaining <0.2 μm fraction are free-form proteinaceous materials produced by bacterial metabolism. TLF can be measured in-situ using ultra-violet fluorimetry and is associated with microbial breakdown of labile, or bioavailable, organic carbon (Elliott et al., 2006; Fox et al., 2017; Hudson et al., 2008). Although labile carbon occurs naturally, faecally contaminated water is characterised by intense TLF peaks that can be identified in contrast to natural baseline levels (Hudson et al., 2007).

This is the first groundwater study to compare TLF with *E. coli* specifically, but previous studies have found it correlated with faecal *Streptococcus* and *Clostridium* bacteria (Lapworth et al., 2008), TTCs in groundwaters (Sorensen et al., 2015a, 2016), *E. coli* in surface waters (Baker et al., 2015; Cumberland et al., 2012), and biological oxygen demand in organic waste streams (Carstea et al., 2016; Hudson et al., 2008). These studies help build a case for the utility of TLF, but do not provide definitive insight into its relationship with pathogens. The relationships between long-used indicators and pathogens remain unclear because direct comparisons are difficult and rare (Ferguson et al., 2012; Sorensen et al., 2015b). In lieu of direct comparisons, one way to consider how TLF and *E. coli* relate to pathogens is by referencing established criteria for an 'ideal' microbial contamination indicator.

The World Health Organization (WHO) stipulates five criteria for indicators, they should 1) be universally present in faeces at higher concentrations than pathogens; 2) persist in the environment and respond to treatment in a similar manner to pathogens but 3) not be pathogenic; 4) be simply and inexpensively detected; and 5) not multiply in natural waters (WHO, 2011). The first criterion is well met by both *E. coli* and TLF. The second, less so; *E. coli* can mimic physiologically similar pathogens but viruses and protozoa have different transport patterns and superior environmental survival times (Leclerc et al., 2001; Osborn et al., 2004). Consequently, the absence of *E. coli* in groundwater does not guarantee its safety. In contrast, TLF in groundwater is strongly associated with <0.2 μm material, potentially giving it a size-based advantage as a more mobile and, therefore, conservative indicator of microbial contamination (Sorensen et al., 2016). There is some evidence that TLF is also more persistent in the environment than culturable TTCs (Sorensen et al., 2015a).

TLF is not specific to any one organism and meets the third criterion of being non-pathogenic. The fourth criterion stipulates simple, inexpensive detection. Typical *E. coli* detection methods rely on a particular enzyme (β-glucuronidase) and require 18 to 48 h, sterile conditions, technical training and a range of consumables. In-situ fluorimetry has much lower variable cost by providing immediate results with minimal process steps, training and consumables.

Finally, the fifth criterion, not multiplying in the environment, is not met by *E. coli* or TLF. That non-*Escherichia coli*forms are present in the environment is a long-standing criticism of their use as indicators (Leclerc et al., 2001). For *E. coli*, many maintain that environmental populations are limited and usually out-competed (WHO, 2012) but studies have reported *E. coli* survival and regrowth within tropical and temperate soils (Brennan et al., 2010a, 2010b; Fujioka et al., 1998; Solo-Gabriele et al., 2000), sediments (Haller et al., 2009), water (Pote et al., 2009), and handpumps (Ferguson et al., 2011). For TLF, natural baseline levels are expected when microbial communities and labile carbon are present, but differentiating between baseline and contaminated conditions is possible because faecal TLF concentrations are high relative to baseline uncontaminated waters (Baker, 2001; Sorensen et al., 2015a).

Although less than ideal, it is widely held that using *E. coli* as an indicator is justified; we do not disagree. We argue that, being well-matched to the discussed criteria, TLF has potential as a complementary parameter. TLF will not replace *E. coli* as an indicator, but it has significant practical advantages for rapid screening and monitoring of microbiological groundwater quality. In this study we investigate the usability and effectiveness of in-situ fluorimetry in comparison with cultured faecal indicator bacteria. Our comparison focusses on agreement and precision of results.

We used two different methods for determining *E. coli* concentrations in our samples and, since other studies of TLF in groundwater have used TTCs for comparison, we also analysed for TTCs to understand how they compare to *E. coli* in our context. Based on comparison with *E. coli* results, we determined thresholds for grouping TLF into corresponding ordinal risk classes. We used paired ordinal logit cumulative link models to assess the level of agreement between the risk classifications generated by the different methods. Our second aim was to determine the relative precision of the methods. We did this by inclusion of duplicates and replicates in our sampling design. Drawing from our findings and experience in the field, as well as the wider literature, we