A critical evaluation of a point-of-care device for measuring intestinal inflammation in field settings

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Introduction

Testing and sharing information about methods that are minimally invasive and field-friendly is important for furthering human biology research. As human biologists, we aim to answer many health-related and evolutionary questions among populations where access to medical care and infrastructure needs (i.e., electricity, cold storage) are limited. Point-of-care devices (i.e., tools that can be used on the spot to assess biomarker levels) are often used to measure blood levels of lipids, glucose, and hemoglobin in field settings from finger-prick blood samples. Beyond these measures, however, little information exists on other available field-friendly point-of-care devices that may be useful in human population biology research.

Further, while point-of-care devices are often validated using gold standard methodologies (e.g., Enzyme-linked immunosorbent assay [ELISA] tests), these validations are never performed in field settings where humidity and temperature may vary from laboratory settings. They also never consider the effects that storage and shipping may have.

Here, we discuss the Bühlmann Quantum Blue Reader (QBR; BUHLMANN Diagnostics Corp., Amherst, NH) – a point-of-care device used to measure Fecal Calprotectin (FC) from stool samples. Fecal Calprotectin is a biomarker of intestinal inflammation and has been shown to be elevated in inflammatory bowel disease[1, 2].

Methods

Stool samples were collected among 24 children (12 boys, 12 girls; ages 5 months through 14 years) in the Mississippi Delta as part of the Rural Embodiment and Child Health (REACH) Study in Summer 2019. Two cryovials of sample were made from each participant and were frozen in a portable freezer throughout the field season (August 2019). One vial was shipped to Global Health Biomarker Lab at the University of Oregon where FC was measured using ELISA (EK-CAL; BULMANN Diagnostics Corp., Amherst, NH; **Figure 1**). The other was shipped to the Human Biology Lab at the University of Colorado Colorado Springs where FC was measured using the QBR (**Figure 2**). In both cases, FC was extracted from the sample using the Calex Cap (B-CALEX-C; BUHLMANN Diagnostics Corp., Amherst, NH; **Figure 3**).

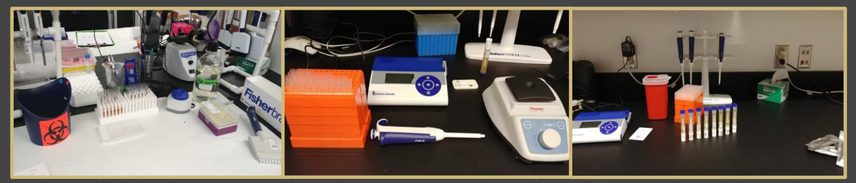

Figure 1 (right): Laboratory set up for analyses using ELISA. **Figure 2 (middle):** Materials needed to analyze FC using the QBR. **Figure 3 (left):** Calex Caps during the extraction process

Research Questions

This study was conducted to discuss methodological considerations and field-friendliness of the QBR. Here, we consider:

1) **How do results obtained from the QBR compare to results obtained via ELISA?** In both cases, samples were collected and processed in a field setting, frozen for a period of time, and shipped to different labs. Does the QBR provide accurate results for answering research questions and for returning information to participants?
2) **How field-friendly is the QBR?** What materials and infrastructure are necessary to use the QBR? Is it useful for conducting research in field settings?

Results

Results from ELISA and QBR were highly positively correlated ($r = 0.931$; $p < 0.001$). **Table 1** contains the results from the ELISA compared to the QBR results. **Figure 4** shows both values for each participant, with lines demonstrating clinically significant elevation levels. In 15 out of 24 cases, the QBR produced higher results than ELISA. It also placed more children in the high elevation category than ELISA did and had one fewer children listed as having no elevation. Based on ELISA, three children were below the limit of detection; two of these were also low based on QBR results, but one was estimated as clinically elevated (FC > 50 ug/g). This, combined with the higher means/median in QBR results compared to ELISA results suggest that the QBR may overestimate FC levels and is less specific than ELISA. While it may be useful for getting at general data trends (due to being closely correlated with ELISA) and for classifying research participants into categories of clinically elevated vs. not (FC < 50 ug/g), the QBR may not be specific enough to examine more nuanced research questions or be used to give accurate information back to participants.

Table 1. Comparison of FC levels and intestinal inflammation categories for all participants

Table 1.	ELISA	QBR
FC Mean (SD; ug/g)	155.15 (121.07)	180.58 (126.02)
FC Median (IQR; ug/g)	139.84 (146.97)	151.00 (189.00)
Intestinal Inflammation		
No Elevation (< 50 ug/g)	4 (16.7%)	3 (12.5%)
Moderate (50-200 ug/g)	14 (58.3%)	12 (50%)
High (>200 ug/g)	6 (25%)	9 (37.5)

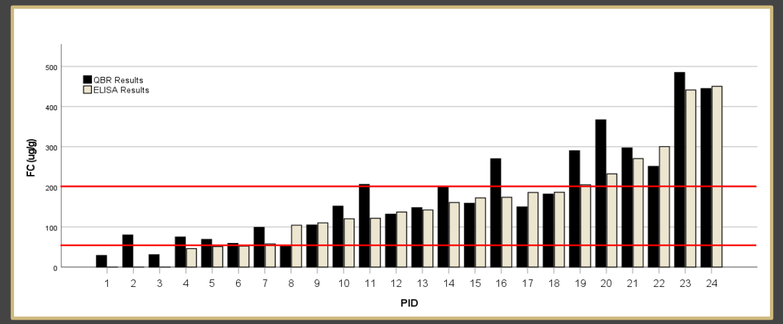

Figure 4. QBR and ELISA results for each participant. Red lines represent the cutoff for moderate (>50 ug/g) and high (>200 ug/g) elevation to show which participants were placed in different categories based on the methods used

Table 2 presents ELISA and QBR data for specific age ranges compared to other samples that have measured FC in children using ELISA. Both the ELISA and QBR results show that children in this sample have very high FC levels and high rates of intestinal inflammation compared to other samples.

It is worth noting that a local nurse practitioner reported high prevalence of active *H. pylori* infection in the community. The children sampled in this study all identified as African American and studies have linked *H. pylori* in African American populations with higher rates of gastric cancers due to variation in inflammatory immune response that could possibly be associated with stress and environmental exposure[3]. These findings suggest a need to further understand relationships between *H. pylori* infection and intestinal inflammation in childhood as a way of understanding risk of gastric cancer in adulthood. These findings also show that FC measured from QBR may be useful in samples where levels are elevated. Further research is needed to examine if the QBR is useful for samples with lower levels of FC.

Table 2. Comparison of age-specific results from this study to similar age-ranges in other studies

	Mean (SD)	Median (SD)	% Elevated
Shuar of Ecuador[1]			
Ages 5-14 years (n = 26)		18	23%
United Kingdom[4]			
Ages 2-9 years (n = 27)	53 (46)	34	
Ages 10-19 years (n = 15)	27 (14)	22	
Norway[2]			
Ages 1-13 years (n = 24)	40 (28)		
Sweden[5]			
Ages 4-6 years (n = 27)		28	
Ages 7-10 years (n = 30)		14	
Ages 11-14 years (n = 27)		10	
Ages 15-17 years (n = 33)		15	
All ages (n = 117)		14	11%
Rural Mississippi ELISA (Present Study)			
Ages 1-3 years (n = 5)	196 (97)	186 (173)	100%
Ages 4-6 years (n = 5)	177 (172)	187 (297)	80%
Ages 7-10 years (n = 9)	121 (69)	120 (91)	78%
Ages 11-14 years (n = 5)	154 (175)	122 (268)	80%
Rural Mississippi QBR (Present Study)			
Ages 1-3 years (n = 5)	207 (95)	251 (174)	80%
Ages 4-6 years (n = 5)	209 (186)	182 (344)	80%
Ages 7-10 years (n = 9)	147 (95)	132 (103)	100%
Ages 11-14 years (n = 5)	186 (159)	148 (261)	80%

While QBR may be useful for comparing levels of FC, there are pros and cons to its use in field settings.

Pros:

- Sampling: The QBR measures FC levels from stool samples, which are minimally invasive and allow participants to collect the samples themselves (or with the help of a parent).
- Power Needs: The device is battery powered, so electricity is not needed, but it should be plugged in to charge.
- Weight/Size: The device is light-weight and can easily be packed for travel. It comes with a sturdy protective case.
- Storage: Calex Caps can be used to extract FC from stool. Extractions can be immediately analyzed, stored for three days at room temperature, or in cold storage for longer periods of time. Unused, the Calex Cap can be kept at room temperature.
- Ease of Use: The QBR gives results within 30 minutes depending on the length of extraction (see cons). The device itself is easy to use and intuitive. While you can run single samples, it is best to wait until you have multiple samples as it will substantially cut down on processing time.

Cons:

- Sampling: Participants may be uncomfortable with the idea of collecting stool samples.
- Power Needs: A vortex, which requires electricity and a stable surface, makes the extraction process easier. While it is listed as optional, extraction using the Calex Caps may be difficult without it.
- Cost: Relatively expensive. The device costs ~$3000. The per participant cost is ~$25. ELISA kits, on the other hand, cost ~$8 per participant.
- Ease of use: Extraction using the Calex Cap can be difficult depending on stool texture. A vortex is necessary in many cases.

Results suggest that the QBR is less specific than ELISA and may overestimate FC levels. It may, however, be useful for looking at general trends and comparing relative levels. It is reasonably field friendly and could be useful for analyzing intestinal inflammation on the spot in environments with limited infrastructure and storage availability.

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References. [1]Cepon-Robins et al. 2019. Soil-transmitted helminth infection and intestinal inflammation among the Shuar of Amazonian Ecuador. [2]Olafsdottir et al., 2002. Faecal calprotectin levels in infants with infantile colic, healthy infants, children with inflammatory bowel disease, children with recurrent abdominal pain and healthy children. [3]Butt et al., 2020. Differences in antibody levels to H. pylori virulence factors VacA and CagA among African Americans and whites in the Southeast USA [4]Joshi et al. 2003. Age-related faecal calprotectin, lactoferrin and tumour M2-PK concentrations in healthy volunteers. [5]Fagerberg et al. 2003. Fecal Calprotectin Levels in Healthy Children Studied With an Improved Assay.