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Development of multiple-micronutrient and environmental enteric dysfunction assessment tool (MEEDAT)

Michael B. Arndt[1,2], Jason L. Cantera[1], Robert K.M. Choy[1], Abby Tyler[3], Christopher Lyman[3], David S. Boyle[1]

[1]PATH, [2]University of Washington, Department of Global Health, [3]Quansys Biosciences

Introduction

- Environmental enteric dysfunction (EED) is an intestinal disorder common among children living in low-resource settings.
- EED is associated with increased risk of growth stunting, cognitive deficits, and reduced responsiveness to oral vaccine.
- Key challenges to diagnosing and treating EED:
  - Lack of validated biomarkers predictive of morbid sequelae.
  - Current tools to quantify biomarkers of gut function and micronutrient status are expensive, time-consuming, and labor-intensive
- Diagnostic need: a tool to quantitate multiple EED biomarkers and micronutrients would increase the efficiency with which children could be screened for EED and micronutrient deficiencies prior to enrollment into clinical trials of candidate EED interventions; this tool would also streamline the evaluation of efficacy.

Objective

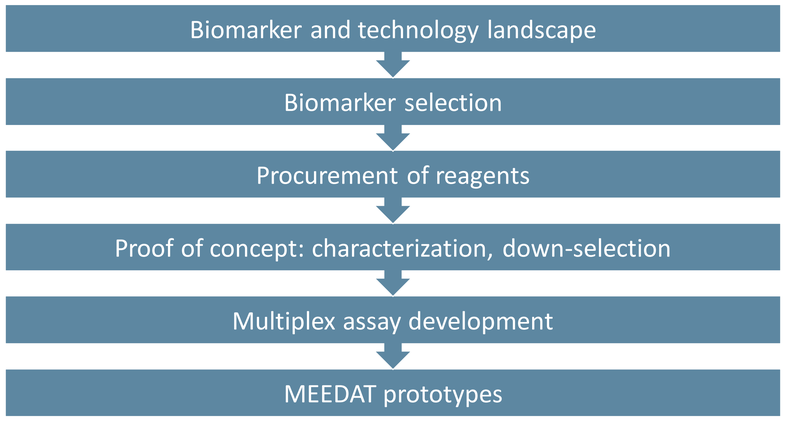
Develop an assay to detect and quantify EED biomarkers in human specimens as a research tool in low-resource settings.

Landscape of EED biomarkers and diagnostic technologies

Matrix	Marker	Current technology	Direction of therapeutic benefit	Domain
Breath	SIBO	Glucose hydrogen breath test	down	Dysbiosis
Plasma	Glucagon-like protein 2	Sandwich ELISA, radioimmunoassay	up	Gut injury/repair
Plasma	I-FABP	Sandwich ELISA	down	Gut injury/repair
Plasma	Citrulline	LC-MS/MS +/- isotope labeling	up	Gut injury/repair
Plasma	Anti-LPS IgA and IgG	Custom ELISA	down	Microbial translocation
Plasma	Anti-FliC IgA and IgG	Custom ELISA	down	Microbial translocation
Plasma	EndoCab	Sandwich ELISA	down	Microbial translocation
Plasma	Soluble CD14	ELISA	down	Microbial translocation
Plasma	IGF-1	Sandwich ELISA	up	Growth hormone status
Plasma	IGFALS	Sandwich ELISA	up	Growth hormone status
Plasma	FGF21	Sandwich ELISA	down	Growth hormone status
Plasma	C-reactive protein	Sandwich ELISA	down	Systemic immune activation
Plasma	α-1-acid glycoprotein	Radial immunodiffusion	down	Systemic immune activation
Plasma	Serum amyloid A protein	Sandwich ELISA	down	Systemic immune activation
Plasma	Pro-inflammatory cytokines	ELISA/multiplex assay (i.e., Luminex®)	down	Systemic immune activation
Plasma	Ferritin	Sandwich ELISA	up	Systemic inflammation/iron
Plasma	Kynurinine	LC-MS/MS	down	Systemic inflammation
Plasma	Tryptophan	LC-MS/MS	up	Systemic inflammation
Plasma	LPS-binding protein	Sandwich ELISA	down	Systemic inflammation
Serum	Zonulin	Semiquantitative: Western blot	down	Gut leakiness
Stool	Microbiota composition	16S ribosomal RNA sequencing	N/A	Dysbiosis
Stool	Myeloperoxidase	Sandwich ELISA	down	Gut inflammation
Stool	Calprotectin	Sandwich ELISA	down	Gut inflammation
Stool	Neopterin	Sandwich ELISA	down	Gut inflammation
Stool	CD53 mRNA transcript	Droplet digital PCR/TaqMan™	down	Gut inflammation
Stool	CDX1 mRNA transcript	Droplet digital PCR/TaqMan™	N/A	Other—cell differentiation
Stool	HLA-DRA mRNA transcript	Droplet digital PCR/TaqMan™	down	Gut inflammation
Stool	MUC12 mRNA transcript	Droplet digital PCR/TaqMan™	down	Gut leakiness—multiple
Stool	REG1A mRNA transcript	Droplet digital PCR/TaqMan™	down	Gut injury/repair
Stool	S100A8 mRNA transcript	Droplet digital PCR/TaqMan™	down	Gut inflammation
Stool	TNF mRNA transcript	Droplet digital PCR/TaqMan™	down	Gut inflammation
Stool	Reg1β	Sandwich ELISA	down	Gut injury/repair
Stool	α-1 antitrypsin	Sandwich ELISA	down	Gut leakiness
Stool	Enteropathogen burden	TaqMan qPCR	down	Other
Urine	Lactulose	LC-MS/MS	down	Gut leakiness
Urine	Claudin-15	Sandwich ELISA	up	Gut leakiness
Urine	Mannitol	LC-MS/MS	up	Nutrient malabsorption
Urine	Rhamnose	LC-MS/MS	up	Nutrient malabsorption

Note: ELISA, enzyme-linked immunosorbent assay; EndoCab, endotoxin-core antibody; CDX1, Caudal Type Homeobox 1; FliC, flagellin; HLA-DRA, HLA class II histocompatibility antigen alpha-chain; I-FABP, intestinal fatty-acid-binding protein; IGFALS, IGF acid labile subunit; LC-MS, liquid chromatography–mass spectrometry; LPS, lipopolysaccharide; mRNA, messenger RNA; MS, ; MUC12, Mucin 12; N/A, not applicable; PCR, polymerase chain reaction; qPCR, quantitative polymerase chain reaction; REG1A/Reg1β, regenerating family member 1- alpha/beta; S100A8, S100 Calcium Binding Protein A8 (calgranulin-A); SIBO, small intestine bacterial overgrowth, TNF, tumor necrosis factor.

Methods



Biomarker selection and rationale

Four plasma markers were selected due to ease of collection/processing, and to employ a low-cost multiplex technology that measures micronutrient status and systemic inflammation (described below).

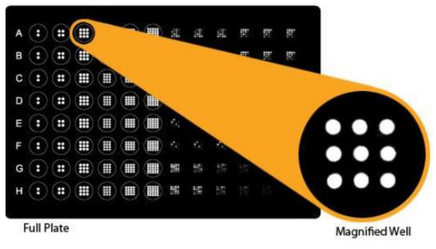
Plasma marker	Full name	Indicates	Associations/mechanism (rationale)[1, 2, 3, 4]	Mean and range* (pg/ml)
I-FABP	Intestinal fatty-acid-binding protein	Small gut injury	•Stunting •Risk of growth faltering	943 0–16,999
GLP-2	Glucagon-like peptide-2	Intestinal regeneration	•Stunting	3,367 0–11,765
sCD14	Soluble CD14	Systemic monocyte activation due to bacterial translocation	•Risk of growth faltering •Poor immune responses to oral immunizations •Future cognition "scores	1,949,857 0–18,738,000
IGF-1†	Insulin-like growth factor 1	Proper function of the growth hormone (GH) axis	•Local binding promotes tissue and bone growth •Low IGF-1 suggests GH resistance	36,796 2,683–84,640

*Estimated from data from pediatric cohorts in Brazil, Bangladesh, and Zimbabwe.
†Alternative biomarkers for IGF-1 included in the proof-of-concept project phase include FGF21 and IGFALS

Platform

Quansys Biosciences Q-Plex™ multiplex assay system:

- Printed antibody arrays efficiently detect multiple analytes; can test up to 20 markers per well using minimal amount of sample.
- Rapid results; assays run in as little as 2 hours.
- Easy-to-use kits, uses standard ELISA protocol/chemiluminescent reporter detection.
- Can analyze 34–40 samples (in duplicate) per plate.
- Low-cost Q-View™ reader and easy-to-use software with all raw and analyzed data in one report.
- Q-Plex™ Human Micronutrient panel (developed by PATH and Quansys) already available and measures AGP, CRP, ferritin, sTfR, RBP4, thyroglobulin, and HRP2[5]



Proof of concept

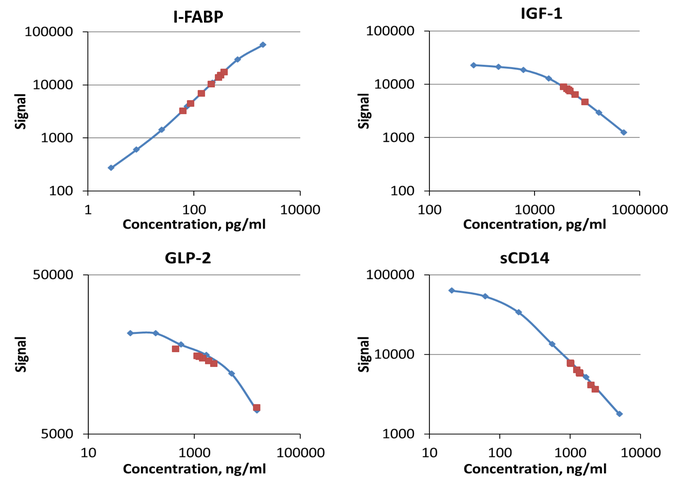


Figure 1. Immunoreagents for I-FABP, IGF-1, GLP-2, and sCD14 were initially evaluated using standard ELISA and further tested in a multiplex assay format. IGF-1, sCD14, and GLP-2 reagents work well using a competitive assay format, and I-FABP works well using a sandwich format. GLP-2 signal is weak as compared with the other biomarkers and requires further optimization. Human samples were tested, as indicated in red.

Summary and conclusions

- Biomarkers for EED have been landscaped and plasma-based markers selected for multiplex assay development.
- The reagents were evaluated in multiplex assay format using the Quansys Biosciences Q-Plex platform.
- Initial results suggest potential to simultaneously detect and analyze the four biomarkers for EED and growth-hormone status.

Next steps

- Develop MEEDAT by multiplexing EED with micronutrient panel biomarkers.
- Conduct lab-based testing of MEEDAT prototypes with clinical specimens to benchmark against commercially available ELISAs.
- Develop user and market requirements documents and target product profile.

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