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Laboratory-Based Performance Evaluation of Multi-Micronutrient and Environmental Enteric Dysfunction Assessment Tool (MEEDAT)

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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 vaccines.
- Key challenges to diagnosing and treating EED:
  - There is a 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.
- PATH has developed a prototype assay to quantitate multiple markers of EED, systemic inflammation, growth hormone (GH) resistance, and micronutrients for use in EED clinical research in low-resource settings.

Objective

Evaluate the performance of the prototype Multi-Micronutrient and EED Assessment Tool (MEEDAT) in quantifying four EED and GH resistance biomarkers in clinical specimens from healthy patients or patients with one of the following: celiac disease, Crohn's disease, pneumonia, GH deficiency, diarrhea, or HIV infection.

Biomarkers of EED and/or child growth in low-resource settings

Matrix	Marker	Current technology	Direction of therapeutic benefit	Domain
Breath	SIBO	Glucose hydrogen breath test	down	Dysbiosis
Plasma	Glucagon-like protein 2	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	GH axis
Plasma	IGFALS	Sandwich ELISA	up	GH axis
Plasma	FGF21	Sandwich ELISA	down	GH axis
Plasma	C-reactive protein	Sandwich ELISA	down	Systemic immune activation
Plasma	α-1-acid glycoprotein	Radial immunodiffusion or ELISA	down	Systemic immune activation
Plasma	Serum amyloid A protein	Sandwich ELISA	down	Systemic immune activation
Plasma	Pro-inflammatory cytokines	ELISA/ Luminex® assay	down	Systemic immune activation
Plasma	Ferritin	Sandwich ELISA	up	Systemic inflammation/iron
Plasma	Kynurenine	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	down	Gut inflammation
Stool	CDX1 mRNA transcript	Droplet digital PCR	N/A	Other—cell differentiation
Stool	HLA-DRA mRNA transcript	Droplet digital PCR	down	Gut inflammation
Stool	MUC12 mRNA transcript	Droplet digital PCR	down	Gut leakiness—multiple
Stool	REG1A mRNA transcript	Droplet digital PCR	down	Gut injury/repair
Stool	S100A8 mRNA transcript	Droplet digital PCR	down	Gut inflammation
Stool	TNF mRNA transcript	Droplet digital PCR	down	Gut inflammation
Stool	REG1β	Sandwich ELISA	down	Gut injury/repair
Stool	α-1 antitrypsin	Sandwich ELISA	down	Gut leakiness
Stool	Enteropathogen burden	Real-time PCR	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; GH, growth hormone; HLA-DRA, HLA class II histocompatibility antigen alpha chain; I-FABP, intestinal fatty-acid-binding protein; IGFALS, insulin-like growth factor acid labile subunit; LC-MS/MS, liquid chromatography–tandem mass spectrometry; LPS, lipopolysaccharide; mRNA, messenger RNA; 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; CD14, cluster of differentiation 14.

Table 1. Landscape of candidate biomarkers (related to EED, systemic inflammation, growth hormone, and/or child health outcomes), matrix, and technology used. Highlighted biomarkers are those included in the MEEDAT panel.

Methods

Biomarker/technology landscape

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Biomarker selection

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Antibody characterization and down-selection

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Multiplex assay development and optimization

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Production of MEEDAT prototypes

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Lab-based performance verification

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Future: Performance validation with infant sera specimens

Biomarker selection and rationale

MEEDAT is an expansion of the Quansys Biosciences Q-Plex™ Human Micronutrient Array (HMA) that measures α1-acid glycoprotein (AGP), C-reactive protein (CRP), ferritin, soluble transferrin receptor (sTfR), retinol binding protein 4 (RBP4), thyroglobulin (Tg), and histidine rich protein 2.[5]

Four plasma markers related to EED or the growth hormone axis were selected due to ease of collection/processing and evidence of relationships with child growth, cognitive development, and oral vaccine immunogenicity.

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
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
FGF21	Fibroblast growth factor 21	Growth hormone (GH) resistance due to reduced protein intake	•Risk of growth faltering during nutritional supplementation	430 25– >2462
IGF-1	Insulin-like growth factor 1	Proper function of the GH axis	•Local binding to receptors 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.

Table 2. Descriptive information, rationale, and previously observed pediatric concentrations for the selected EED or GH axis biomarkers.

Performance verification

- Procurement of de-identified sera specimens (N = 48) from US-based healthy patients or patients with celiac disease, Crohn's disease, pneumonia, GH deficiency, diarrhea, or HIV infection from commercial biorepositories.
- Testing of each diluted specimen using both the prototype MEEDAT and standard biomarker-specific immunoassays to measure the levels of the four new biomarkers.
- Comparison of the results from the MEEDAT with those obtained by standard immunoassay methods for each respective biomarker via Pearson correlation.
- Evaluation of concentration-dependent bias in differences between measurements from the MEEDAT and monoplex ELISAs using Bland-Altman plots with the average concentration value for the two methods (x-axis) plotted against the difference in concentration (y-axis).

Results

Marker	I-FABP (pg/ml)	sCD14 (ng/ml)	FGF21 (pg/ml)	IGF-1 (ng/ml)
Median	844	1,901	111	108
Range	218–5,395	877–6,638	10–2,157	35–404

Table 3. Descriptive statistics for the selected EED and GH resistance biomarkers as measured by the MEEDAT prototype in the 48 sera specimens.

Results

Marker	I-FABP	sCD14	FGF21	IGF-1	CRP	AGP	RBP4	Ferritin	Tg	sTfR
Rho	0.920	0.710	0.945	0.854	0.857	0.390	0.881	0.994	0.780	0.600

Table 4. Pearson correlation coefficients for biomarkers (MEEDAT vs. monoplex). Monoplex data for ferritin, Tg, sTfR, CRP, AGP, and RBP4 are from specimens (n = 10) tested previously with HMA and monoplex ELISAs.

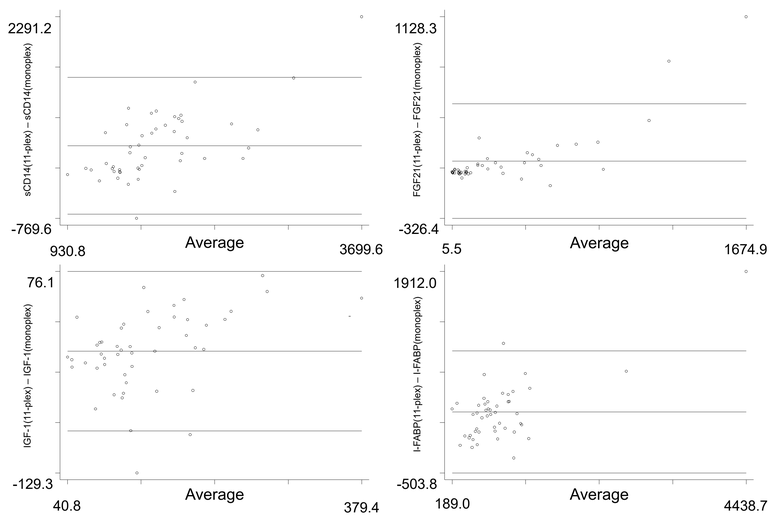

Figure 1. Bland-Altman plots comparing 11-plex to monoplex for sCD14 (ng/ml), FGF21 (pg/ml), IGF-1 (ng/ml), and I-FABP (pg/ml).

- Bland-Altman plots do not suggest that the absolute difference between the two assays (y-axes) increases or decreases as the average value (x-axes) increases.
- There is a paucity of data in the upper half of the range on the x-axis for all of the analytes; this is especially true for I-FABP.
- Poor correlation for AGP most likely due to sample degradation.

Conclusions

- The performance of a multiplex Q-Plex immunoassay was evaluated for its ability to quantify four EED and GH biomarkers in clinical sera specimens.
- Strong correlation between the concentration results were obtained from MEEDAT and monoplex ELISAs for each of the four new biomarkers.
- Minimal evidence of concentration-dependent bias in the differences between measurements for each biomarker was obtained from the two determination methods.
- Prototype MEEDAT was able to simultaneously detect and quantify the four biomarkers for EED and GH status in addition to those biomarkers on the HMA.
- A larger data set with more extreme values (e.g., from children in low-resource settings) is needed to fully validate the performance of MEEDAT for new biomarkers.

Next steps

- Performance validation on archived sera specimens from Malian infants who were enrolled in an oral rotavirus vaccine booster trial.
- Test feasibility of MEEDAT use in EED clinical trials: MEEDAT testing of baseline and follow-up sera samples from children enrolled in a phase I randomized controlled trial of amino-acid based oral rehydration solution in Western Kenya.
- Use in future observational pediatric research studies in Mali, Kenya, and Laos.
- Potential expansion to include additional putative EED biomarkers.
- Potential for broader application in nutrition surveillance programs.

Acknowledgments

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