# CHAPTER 4.

# SYSTEMATIC REVIEW OF EED BIOMARKERS/DIAGNOSTIC TESTS: RESULTS SYNOPSIS



Denno, DM, VanBuskirk, KM, Nelson, ZC, Musser, CA, Tarr, PI. (2016). Environmental enteric dysfunction: Advancing current knowledge. St. Louis, MO: Washington University Libraries. http://dx.doi.org/10.7936/K7WQ0228

# Chapter 4. Systematic Review of EED Biomarkers/Diagnostic Tests: Results Synopsis

## 4.1 Biomarkers and Diagnostics Systematic Search Results

The query of our EED Library to identify references potentially relevant to the systematic review produced 361 citations for the time period between 2000 and 2010. The "snowball" technique identified 13 additional potentially relevant publications that were not found through the original systematic search. Thirty-three of the 374 potentially relevant publications were in languages other than those that we included for this review—English, French, German, Italian, Portuguese, and Spanish (Table 8).

**Table 8. Breakdown of publications in excluded languages of potential relevance to the systematic review.**

| Language | Number potentially relevant | Number sufficiently reviewed and excluded for reasons other than language |
|---|---|---|
| Arabic | 1 | 0 |
| Chinese | 3 | 1 |
| Croatian | 2 | 2 |
| Czech | 2 | 1 |
| Norwegian | 1 | 0 |
| Polish | 14 | 10 |
| Russian | 9 | 6 |
| Turkish | 1 | 1 |
| **Totals** | **33** | **21** |

However, we were able to determine (via translation and/or English language abstract) that 21 of the 33 did not meet our other systematic review inclusion criteria. We were unable to sufficiently translate the remaining 12 papers to determine their relevance or, if they were relevant, to extract data. These papers were in Arabic (n=1), Chinese (n=2), Czech (n=1), Norwegian (n=1), Polish (n=4), and Russian (n=3). Two-hundred and fifty publications were eliminated for various reasons, including their focus on celiac disease, cow's milk protein allergy, or inflammatory bowel disease; lack of at least three subjects of interest under five years of age; or they did not assess biomarkers or diagnostic tests related to small intestinal function or inflammation. One potentially relevant article was thought to be pertinent to the systematic review based on the abstract, and another article required full text for further determination; however, the full texts for these two articles could not be found.

An additional 12 references were highly considered for inclusion, but ultimately excluded from the review for reasons including insufficient subjects in the specified age range, inadequate relevant data on appropriately-aged subjects, or uncertainty about the setting in which the study was performed (Appendix 5).

We identified 20 review articles with content that addressed or discussed material of relevance to our systematic review question (Appendix 6); however, we identified no systematic review that had been conducted on biomarkers or diagnostic tests related to EED.

# 4.2 Characteristics of References Included in the Systematic Review

The remaining 77 references included in the systematic review (Table 9) describe research that was performed in 22 different countries. Figure 7 maps the sites where the studies were performed.

**Table 9. Articles pertinent to the systematic review.**

These were determined to be the publications of interest between 2000 and 2010.

| |
|---|
| Alcantara CS, et al. Interleukin-8, tumor necrosis factor-alpha, and lactoferrin in immunocompetent hosts with experimental and Brazilian children with acquired cryptosporidiosis. 2003. [101] |
| Alves GM, et al. Nutritional status and breath hydrogen test with lactose and lactulose in Terena Indian children. 2002. [102] |
| Amadi B, et al. Reduced production of sulfated glycosaminoglycans occurs in Zambian children with kwashiorkor but not marasmus. 2009. [103] |
| Azim T, et al. Immune response of Bangladeshi children with AD who subsequently have persistent diarrhea. 2000. [104] |
| Bhatnagar S, et al. Celiac disease with mild to moderate histological changes is a common cause of chronic diarrhea in Indian children. 2005. [105] |
| Bitarakwate E, et al. Serum zinc status of children with persistent diarrhoea admitted to the diarrhoea management unit of Mulago Hospital, Uganda. 2003. [106] |
| Bukhari AS, et al. DNA damage and plasma homocysteine levels are associated with serum metabolites and mineral constituents' profiles in children with persistent diarrhea. 2010. [107] |
| Bushen OY, et al. Heavy cryptosporidial infections in children in northeast Brazil: comparison of *Cryptosporidium hominis* and *Cryptosporidium parvum*. 2007. [108] |
| Bustos M, et al. Disaccharidase deficiency in Bolivian children with persistent diarrhea. 2003. [109] |
| Campbell DI, et al. Intestinal inflammation measured by fecal neopterin in Gambian children with enteropathy: association with growth failure*, Giardia lamblia*, and intestinal permeability. 2004. [15] |
| Campbell DI, et al. Growth faltering in rural Gambian infants is associated with impaired small intestinal barrier function, leading to endotoxemia and systemic inflammation. 2003. [110] |
| Campbell DI, et al. Chronic T cell-mediated enteropathy in rural west African children: relationship with nutritional status and small bowel function. 2003. [111] |
| Campbell DI, et al. Age-related association of small intestinal mucosal enteropathy with nutritional status in rural Gambian children. 2002. [112] |
| Chen P, et al. Association of vitamin A and zinc status with altered intestinal permeability: analyses of cohort data from northeastern Brazil. 2003. [113] |

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|---|
| Clark TD, et al. Risk factors and cumulative incidence of anaemia among human immunodeficiency virus-infected children in Uganda. 2002. [114] |
| Darboe MK, et al. Effectiveness of an early supplementation scheme of high-dose vitamin A versus standard WHO protocol in Gambian mothers and infants: a randomised controlled trial. 2007. [115] |
| Dini E, et al. Sudan III and steatocrit in the detection of fecal fat in malnourished children. 2002. [116] |
| El Mouzan MI, et al. Endoscopic duodenal biopsy in children. 2006. [117] |
| Fagundes-Neto U, et al. Studies of the small bowel surface by scanning electron microscopy in infants with persistent diarrhea. 2000. [118] |
| Filteau SM, et al. The effect of antenatal vitamin A and (beta)-carotene supplementation on gut integrity of infants of HIV-infected South African women. 2001. [119] |
| Galpin L, et al. Effect of *Lactobacillus* GG on intestinal integrity in Malawian children at risk of tropical enteropathy. 2005. [120] |
| Gandolfi L, et al. Antiendomysial antibody test reliability in children with frequent diarrhea and malnutrition: is it celiac disease? 2001. [121] |
| Goto R, et al. Impact of anti-*Giardia* and anthelminthic treatment on infant growth and intestinal permeability in rural Bangladesh: a randomised double-blind controlled study. 2008. [122] |
| Goto R, et al. Impact of intestinal permeability, inflammation status and parasitic infections on infant growth faltering in rural Bangladesh. 2008. [123] |
| Goto R, et al. Poor intestinal permeability in mildly stunted Nepali children: Associations with weaning practices and *Giardia lamblia* infection. 2002. [124] |
| Haase A, et al. Dual sugar permeability testing in diarrheal disease. 2000. [125] |
| Hafeez A, et al. An audit of pediatric upper gastrointestinal endoscopies. 2000. [126] |
| Jain S, et al. Fecal occult blood screening in children with severe malnutrition. 2007. [127] |
| Kapoor L, et al. Giardiasis--clinical and diagnostic perspective. 2001. [128] |
| Kapoor S, et al. Detecting protein losing enteropathy by Tc-99m dextran scintigraphy: A novel experience. 2002. [129] |
| Kirkpatrick BD, et al. Serum mannose-binding lectin deficiency is associated with cryptosporidiosis in young Haitian children. 2006. [130] |
| Kirkpatrick BD, et al. Childhood cryptosporidiosis is associated with a persistent systemic inflammatory response. 2006. [131] |
| Kirkpatrick BD, et al. Cryptosporidiosis stimulates an inflammatory intestinal response in malnourished Haitian children. 2002. [132] |
| Kohli A, et al. *Giardia duodenalis* assemblage, clinical presentation and markers of intestinal inflammation in Brazilian children. 2008. [133] |
| Kukuruzovic R, et al. Increased nitric oxide production in AD is associated with abnormal gut permeability, hypokalemia and malnutrition in tropical Australian aboriginal children. 2003. [43] |
| Kukuruzovic R, et al. Milk formulas in acute gastroenteritis and malnutrition: a randomized trial. 2002. [134] |
| Kukuruzovic R, et al. Small bowel intestinal permeability in Australian aboriginal children. 2002. [58] |
| Laadhar L, et al. Determination of anti-transglutaminase antibodies in the diagnosis of celiac disease in children: results of a five year prospective study. 2004. [135] |
| Leite CA, et al. Functional, microbiological and morphological intestinal findings among human immunodeficiency virus infected children. 2006. [136] |

Lima AA, et al. Effects of vitamin A supplementation on intestinal barrier function, growth, total parasitic, and specific *Giardia* spp infections in Brazilian children: a prospective randomized, double-blind, placebo-controlled trial. 2010. [137]

Lima AA, et al. Intestinal barrier function and weight gain in malnourished children taking glutamine supplemented enteral formula. 2005. [138]

Lima NL, et al. Wasting and intestinal barrier function in children taking alanyl-glutamine-supplemented enteral formula. 2007. [139]

Long KZ, et al. The effect of vitamin A supplementation on the intestinal immune response in Mexican children is modified by pathogen infections and diarrhea. 2006. [140]

López de Romaña D, et al. Longitudinal measurements of zinc absorption in Peruvian children consuming wheat products fortified with iron only or iron and 1 of 2 amounts of zinc. 2005. [141]

Mahmud MA, et al. Sociodemographic, environmental and clinical risk factors for developing persistent diarrhea among infants in a rural community of Egypt. 2001. [142]

Mahmud MA, et al. Increased fecal IgE among infants in a rural community of Egypt: an analysis of associated risk factors. 2001. [143]

Manary ML, et al. Zinc homeostasis in Malawian children consuming a high-phytate, maize-based diet. 2002. [144]

Mishra OP, et al. Endoscopic and histopathological evaluation of preschool children with chronic diarrhea. 2001. [145]

Mittal SK, et al. Tropical Sprue in North Indian Children. 2001. [146]

Moya-Camarena SY, et al. Effects of asymptomatic *Giardia intestinalis* infection on carbohydrate absorption in well-nourished Mexican children. 2002. [147]

Murphy JL, et al. Maldigestion and malabsorption of dietary lipid during severe childhood malnutrition. 2002. [148]

Murphy JL, et al. Gastrointestinal handling and metabolic disposal of 13C-labelled tripalmitin during rehabilitation from childhood malnutrition. 2001. [149]

Nichols B, et al. Contribution of villous atrophy to reduced intestinal maltase in infants with malnutrition. 2000. [53]

Northrop-Clewes CA, et al. Anthelmintic treatment of rural Bangladeshi children: effect on host physiology, growth, and biochemical status. 2001. [150]

Panter-Brick C, et al. Pathways leading to early growth faltering: An investigation into the importance of mucosal damage and immunostimulation in different socio-economic groups in Nepal. 2009. [151]

Perin NM, et al. Intestinal absorption of D-xylose in children infected with the human immunodeficiency virus. 2001. [152]

Pires AL, et al. Digital morphometric and stereologic analysis of small intestinal mucosa in well-nourished and malnourished children with persistent diarrhea. 2003. [153]

Poddar U, et al. Is tissue transglutaminase autoantibody the best for diagnosing celiac disease in children of developing countries? 2008. [154]

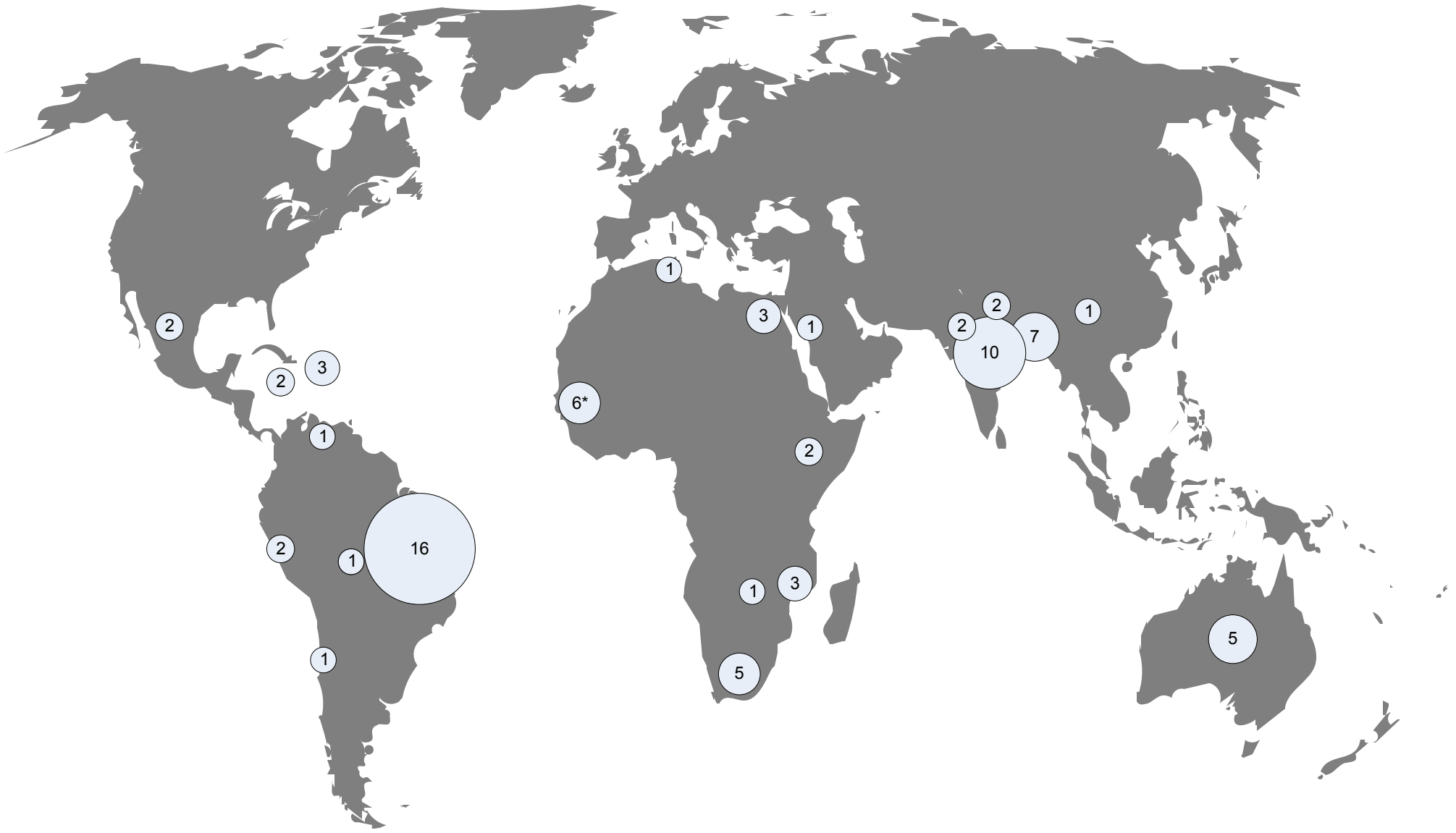
Poddar U, et al. Celiac disease in India: Are they true cases of celiac disease? 2002. [155]

Quadro L, et al. Retinol and retinol-binding protein: gut integrity and circulating immunoglobulins. 2000. [156]

Rabbani GH, et al. Green banana and pectin improve small intestinal permeability and reduce fluid loss in Bangladeshi children with persistent diarrhea. 2004. [157]

| |
|---|
| Rabbani GH, et al. Increased nitrite and nitrate concentrations in sera and urine of patients with cholera or shigellosis. 2001. [158] |
| Ritchie BK, et al. 13C-sucrose breath test: novel use of a noninvasive biomarker of environmental gut health. 2009. [159] |
| Rollins NC, et al. Feeding mode, intestinal permeability, and neopterin excretion: A longitudinal study in infants of HIV-infected South African women. 2001. [160] |
| Rollins NC, et al. Vitamin A supplementation of South African children with diarrhea: optimum timing for improving biochemical and clinical recovery and subsequent vitamin A status. 2000. [161] |
| Samie A, et al. *Cryptosporidium* species: preliminary descriptions of the prevalence and genotype distribution among school children and hospital patients in the Venda region, Limpopo Province, South Africa. 2006. [162] |
| Sarker SA, et al. *Helicobacter pylori* infection, iron absorption, and gastric acid secretion in Bangladeshi children. 2004. [163] |
| Sheng XY, et al. Major variables of zinc homeostasis in Chinese toddlers. 2006. [164] |
| Sherwani K, et al. Prevalence of iron deficiency anemia in chronic diarrhoea and celiac disease - A western UP experience. 2008. [165] |
| Soliman SM, et al. Role of micronutrient mixture in acute and persistent diarrhea in infants and its impact on nutritional status. 2003. [166] |
| Tassara O, et al. Gastrointestinal diseases in children infected with the human immunodeficiency virus. 2003. [167] |
| Thurnham DI, et al. Innate immunity, gut integrity, and vitamin A in Gambian and Indian infants. 2000. [168] |
| Trehan I, et al. A randomized, double-blind, placebo-controlled trial of rifaximin, a nonabsorbable antibiotic, in the treatment of tropical enteropathy. 2009. [13] |
| Vieira MM, et al. Carotenoids, retinol, and intestinal barrier function in children from northeastern Brazil. 2008. [169] |
| Williams EA, et al. A double-blind, placebo-controlled, glutamine-supplementation trial in growth-faltering Gambian infants. 2007. [170] |
| Willumsen JF, et al. Subclinical mastitis as a risk factor for mother-infant HIV transmission. 2000. [171] |
| Zhang Y, et al. Lactulose-mannitol intestinal permeability test in children with diarrhea caused by rotavirus and *Cryptosporidium*. 2000. [172] |

Figure 7. Geographic mapping of study sites
Number of studies by country included in the systematic review.




*One of the studies attributed only to The Gambia in this map had two study locations: The Gambia and India.

Aggregated characteristics of the included studies are presented in Table 10. Details of the data from each article are reported in the Evidence Table of all studies included in the review (Appendix 7). Data are also presented in individual Evidence Tables based on category of biomarker (Evidence Tables 1-8). It is important to note that we only include data from articles that pertain to our review question. No identified study was explicitly designed to assess the accuracy of the diagnostic tests or biomarkers that they employed among children in developing-country settings. While this does not detract from their intrinsic value, it does pose an additional challenge to our goal of evaluating diagnostics for EED. For example, we did not find data pertaining specifically to standard measures of diagnostic test evaluation, such as positive or negative predictive values, or receiver operating characteristic curves. We assessed the use of the biomarkers as they were employed and extracted data relevant to the markers themselves, even if these data were not the primary focus of the studies.

**Table 10. Overview of studies.**

Characteristics of studies are provided and demonstrate broad-based nature of current and recent data.

| Study sites by WHO regions, countries: Number of studies | | |
|---|---|---|
| | WPRO: China (1), Australia (5)<br>AMRO: Brazil (16), Haiti (3), Mexico (2), Peru (2), Venezuela (1), Chile (1), Bolivia (1), Jamaica (2)<br>AFRO: Zambia (1), Malawi (3), The Gambia (6), South Africa (5), Uganda (2)<br>EMRO: Saudi Arabia (1), Pakistan (2), Egypt (3), Tunisia (1)<br>SEARO: India (10), Bangladesh (7), Nepal (2) | |
| Publication year: Number of studies | | |
| | 2000:10<br>2001:12<br>2002:13<br>2003:10<br>2004:4<br>2005:4 | 2006:7<br>2007:5<br>2008:7<br>2009:3<br>2010:2 |
| Language of Publication: Number of studies | | |
| | French: 1<br>Portuguese: 2<br>Spanish: 3<br>English: 71 | |

| | |
|---|---|
| Study durations: Time period for subject enrollment | |
| | Median: 502 days<br>Range: 28 days – 17 years<br>Not specified: 27 studies |
| Publication Lag: Time from study enrollment to publication | |
| | Median: 4 years<br>Range: 1 year – 15 years<br>Not specified: 37 studies |
| Study setting: Number of studies | |
| | Urban: 49<br>Peri-urban: 5<br>Rural: 16<br>Urban slum[1]: 7 |
| Study designs: Number of studies | |
| | Case-controls: 25<br>Longitudinal cohort: 14<br>Randomized controlled trial: 16<br>Cross-sectional: 12<br>Case-series: 10 |
| Sample size of subjects of interest: Number of subjects in all studies combined | |
| | Total: 7730<br>Interquartile Range: 104<br>Median: 75<br>Range: 3-318 |
| Age range of subjects investigated in all studies combined | |
| | Range: birth-88 years |
| Subjects of interest to this review under five years of age: Number of subjects all studies combined | |
| | Total: 5419[2]<br>Interquartile range: 119<br>Median: 71<br>Range: 3-306 |
| Presenting conditions of study subjects of interest at the time of recruitment: Number of studies[3] | |
| | Acute Diarrhea: 27<br>Persistent Diarrhea: 30<br>Infection with specific enteric pathogens: 28<br>*Cryptosporidium* (8)<br>*Giardia* (11)<br>Helminths (4)<br>Helicobacter pylori (3)<br>Other (13)<br>Malnutrition: 45<br>Infected with HIV or Tuberculosis: 9<br>Healthy / asymptomatic: 40<br>Other: 18 |

[1] This comprises any study noted as being conducted in a slum, shantytown, or urban squatter settlement.
[2] Excluding 22 studies that do not specify subjects ≤4 and 5 years.
[3] Number >77 because some studies included more than one condition or test.

Types of specimens and biomarkers reported: Number of studies[4]

| | | |
|---|---|---|
| Blood (25)<br>Hemoglobin: 10<br>Albumin: 9<br>Immunoglobulins: 7<br>Lactulose:rhamnose ratio (L:R): 5<br>α-1-acid glycoprotein: 4<br>CRP: 5<br>Red blood cell indices (e.g., Mean corpuscular volume): 4<br>WBC: 4<br>Total protein: 3<br>D-xylose: 2<br>Transferrin (saturation): 2<br>α-1-antichymotrypsin: 2<br>Cytokines: 1<br>Immune function assays: 1<br>Mannose-binding lectin: 1<br>Nitric oxide: 1<br>Oxidative stress markers, DNA damage to lymphocytes, liver enzymes, thyroid hormones: 1<br>Radiolabeled iron challenge: 1<br><br>Breath (4)<br>$^{13}$C lipid breath test: 1<br>$^{13}$C sucrose breath test: 1<br>Hydrogen breath test<br>Lactose: 2<br>Lactulose: 1<br>Xylose: 1 | Urine (32)<br>Lactulose:mannitol (L:M): 25[5]<br>Radiolabeled zinc or lipid challenge: 3<br>Neopterin: 2<br>Nitric oxide: 2<br>Sucrose:lactulose ratio: 2[6]<br>D-xylose: 1<br>Lactose:lactulose ratio: 1[7]<br>Lactulose:rhamnose (L:R): 1<br>Sucralose:lactulose ratio: 1[8]<br><br>Stool (24)<br>Lactoferrin: 9<br>Leukocytes: 4<br>Cytokines: 5<br>Fecal fat: 3<br>Radiolabeled zinc or lipid challenge: 4<br>Reducing substances: 4<br>Occult blood/RBCs by microscopy: 3<br>IgE: 2<br>Neopterin: 1 | Small intestinal aspirates (2)<br>Concentrations of immunoglobulins (Ig) IgA, IgG, and IgM: 1<br>Microbial concentrations: 1<br><br>Small intestinal endoscopic gross visualization (4)<br><br>Small intestinal tissue (18)<br>Histopathology: 17<br>Disaccharidases: 2<br>Protein and inflammatory markers: 2<br>Messenger RNA abundances: 1<br><br>Site not specified (2)<br>D-xylose: 2 |

For studies that included small intestinal biopsy, characteristics specific to biopsy results:

Total subjects of interest: 996

Subjects of interest under five years of age: 8 studies specified (n=311), 10 did not specify

Site of small intestinal biopsy: Number of studies
- Duodenum: 11
- Jejunum or ileum: 4
- Not specified: 3

Esophagus or stomach also biopsied: 1
Large intestine or rectum also biopsied: 2

[4] Number >77 because some studies included more than one condition or test.
[5] 18 of the 25 studies include fractional excretion of the individual components.
[6] Includes fractional excretion of the individual components.
[7] Includes fractional excretion of the individual components; there was also one study that measured lactose excretion individually without relationship to another sugar.
[8] Includes fractional excretion of the individual components.

| Comparison of biomarkers to histopathology: Number of studies | |
|---|---|
| | Yes: 3 studies compared extra-intestinal tissue markers to histopathology: D-xylose (2)[9]; Fecal fat (1); L:M (1)[10]<br><br>Yes: 3 studies compared endoscopic visualization or intestinal tissue markers to histopathology: Intestinal maltase activity and various intestinal mRNA abundances (1), endoscopic gross visualization (1), scanning electron microscopy (compared to light microscopy) (1)<br><br>No: 71 studies |
| Comparison of extra-intestinal tissue biomarkers to other extra-intestinal tissue biomarkers: Number of studies | |
| | Yes: 12 studies[11] comparing:<br>L:M vs. albumin (1), Immunoglobulins (2), Alpha-1-acid glycoprotein AGP (1), Endotoxin and IgG endotoxin core antibody (1) Fecal neopterin (1); Lactose and Lactose:lactulose (1)<br><br>Serum L:R vs. urinary L:R (1), sucrose breath test (1), urinary nitric oxide (1), serum lactose (1), reducing substances (1), red cell indices (1)<br>Fecal lactoferrin vs. TNF-α receptor I (1), Urinary lactose:creatinine vs. hemoglobin (1); Urinary nitrites vs. stool reducing substances (1); Serum nitrites vs. WBC (1)<br><br>No: 65 studies |

We calculated that in the 77 papers analyzed, a total of 5,410 children under five years of age were studied for any biomarker plausibly related to EED, and an additional 2,311 children were studied, among whom the number of subjects aged under five years could not be determined (Table 10). More than 50 different biomarkers were studied. These biomarkers were obtained by study of urine, stool, blood, breath, and intestinal tissue. Eighteen studies examined the histopathology of biopsied intestinal tissue, but only three studies compared intestinal histopathology to non-intestinal tissue biomarkers (D-xylose; fecal fat; urinary L:M [111, 136, 155]). One additional study compared results of intestinal tissue markers (maltase activity and intestinal mRNA abundances for various markers) to histopathology [53]. Notably, few small

[9] One study compared D-xylose as well as fecal fat.
[10] This study compared the lactulose:mannitol ratio (L:M) to morphometric analysis of biopsy tissue.
[11] Number of comparisons listed is >12 because some studies included more than one comparison.

bowel biopsies (the “gold standard” diagnostic) among children under five years of age in developing-country settings are included in this cohort of studies from the past eleven years. Indeed, small bowel biopsies from only 311 children under five years of age in eight studies were reported, and an additional 685 children in 10 other studies had small bowel biopsies but the number of those who were under five years of age could not be determined.

We were also interested in the lag time between study enrollment and publication, and examined a subset of the first 24 articles relevant to the systematic review. Table 11 provides intervals between enrollment, study start and close, study duration, and year of publication for a subset of the first 24 articles that we reviewed. There were, in general, long delays in cohort enrollment and dissemination of primary data (up to 19 years, and often over a decade).

**Table 11. Study timing analysis.**

Dates of publication, enrollment, and performance of a subset of publications. Seven of 24 studies did not specify study time interval.

| Publication Year | Study enrollment years | Range of study intervals |
|---|---|---|
| 2006 | Aug 1994-May 1995 | 1991-2002 |
| | 30 days – date not specified | |
| | 1993-2002 | |
| | Not specified | |
| | 9 months – date not specified | |
| | 15 months – date not specified | |
| | Not specified | |
| | 1991-2001 | |
| 2007 | Sept 2001-October 2004 | 2001-2004 |
| | July 2003-November 2004 | |
| | 5 years – date not specified | |
| 2008 | June 2003-February 2004 | 1989-2004 |
| | June 2003-April 2004 | |
| | Jan 2000-Dec 2002 | |
| | Not specified | |
| | Aug 1989-Apr 1993 (current study 2004) | |
| | July 2000-Aug 2001 | |
| 2009 | 3 days- date not specified | 1998-2007 |
| | Date not specified | |
| | 2005 – 7 months | |
| | Sept 2007 | |
| | 1998-2000 | |
| 2010 | June 2000-Aug 2004 | 2000-2004 |
| | Not specified | |

# 4.3 Classification of Biomarkers and Diagnostic Tests

The “job description” of the small bowel can be reduced to a circumscribed set of tasks: break down specific nutrients using enzymes in the intestinal lining or by micellization of lipophilic substances; extract nutrients from food; exclude the food components that have no nutritive value and propel them distally for elimination; retain (i.e., not leak into the gut) molecules used (and often synthesized) by the host; and resist microbial breach of the barrier. Hence, the intestinal mucosa serves as a specialized transporting organ as well as a physical, physiologic, and antimicrobial interface between the host and the environment which in this case consists of ingested food and water, but also other potentially highly contaminated materials (e.g., soil) [173], given the oral-motor activities of infants and toddlers. The cells and submucosa that line the small bowel are, therefore, critical for assimilation of nutrients, maintenance of structural integrity, and protection against microbial assault. In injury, substances are absorbed by barrier breach rather than by physiologic transport or diffusion.

We strove to take an unbiased and uniform approach to classifying the EED markers. Our classification system was blinded to the assertions of study authors regarding marker category; however we did find that investigators’ categorizations were largely consistent with ours. Our classification system was based on the primary function/dysfunction that the test is likely to measure or the underlying pathophysiology and pathogenesis that the test may likely reflect. For example, the amount of D-xylose that is absorbed after an oral challenge is believed to reflect gut absorptive capacity, and D-xylose uptake from the challenge, measured either in the blood or urine, is therefore classified as a test of gut absorption. We also aimed to unambiguously place a marker in one group based on best fit when possible, though we recognize that markers might detect derangements of multiple functions. For example, the

presence of lactose in the blood or urine likely indicates a loss of lactase enzyme in the intestinal brush border, and thus can be a marker of abnormal digestion or nonspecific intestinal injury. However, for lactose to traverse the mucosa and gain access to the systemic circulation, a porosity defect is needed. Hence, we chose to place this marker in the permeability category.

Finally, we recognize that many tests reflect nonspecific injury and processes, which cannot be so easily binned into mechanistic or pathophysiologic categories. For example, while measures of surface area on a biopsy provide a general impression of absorptive capacity, we categorize histopathology as a measure of nonspecific injury, as the visualization of tissue portrays a general picture of derangements in architecture without specifying function.

With these factors in mind, we formulated eight test categories, classified in Table 12, and constructed evidence tables based on these classifications.

**Table 12. Classification framework for biomarkers of intestinal function/dysfunction and inflammation.**

| Evidence Table | Type of Functional Measure |
|---|---|
| 1 | Absorption |
| 2 | Porosity/permeability (with or without assessment of absorption) |
| 3 | Digestion |
| 4 | Intestinal inflammation and/or intestinal immune activation |
| 5 | Systemic inflammation and/or systemic immune activation |
| 6 | Microbial drivers |
| 7 | Nonspecific intestinal injury |
| 8 | Non-small intestine organ function |