Supplemental Table 1: Biomarkers measured between 6 and 18 weeks of age.

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| --- | --- | --- | --- | --- | --- | --- |
| Biomarker | Description | Child Age (weeks) | Sample type | Analytical Grouping (Indicative of) | Assay | MeanS.E or Percentage |
| MPO | Myeloperoxidase; component of the neutrophil cytosol | 12 | Stool | Enteric inflammation | ELISA  (ALPCO) | 10979.0211770.10  ng/ml |
| Calprotectin | Component of the neutrophil cytosol | 12 | Stool | Enteric inflammation | ELISA  (ALPCO) | 794.15741.65  ug/g |
| Neopterin | Produced by stimulated monocytes/macrophages | 12 | Stool | Enteric inflammation | ELISA  (Genway Biotech) | 2783.552730.51  nmol/l |
| Alpha-1 anti-trypsin | Plasma protease inhibitor. Presence in stool indicative of gut barrier dysfunction | 12 | Stool | Enteric inflammation | ELISA  (ALPCO) | 0.860.70  mg/g |
| Mannitol | Monosaccharide administered orally and measured in urine as an indicator of gut absorptive capacity | 12 & 24 | Urine | Enteric inflammation | High performance ion chromatography1 | 0.020.02  0.020.02 |
| Reg1β | Regeneration factor, produced by intestinal epithelial cells | 6 & 12 | Stool | Enteric inflammation | ELISA  (TechLab) | 57.7197.71  79.46115.74  ug/ml |
| Days of diarrhea | Number of days of diarrhea by 18 weeks of age | 186 | Active Surveillance2 | Enteric inflammation | N/A | 6.3511.67  days |
| IL-1β | Inflammatory cytokine produced by monocytes and macrophages | 18 | Plasma | Systemic inflammation | Bioplex assay3 (Biorad) | N/A |
| IL-4 | Induces differentiation to Th2 cells | 18 | Plasma | Systemic Inflammation | Bioplex assay3 (Biorad) | N/A |
| IL-5 | Growth factor for B cells and eosinophils | 18 | Plasma | Systemic inflammation | Bioplex assay3 (Biorad) | N/A |
| IL-6 | Inflammatory cytokine produced during acute and chronic inflammation | 18 | Plasma | Systemic inflammation | Bioplex assay3  (Biorad) | N/A |
| IL-7 | B and T cell development and maturation | 18 | Plasma | Systemic Inflammation | Bioplex assay3 (Biorad) | N/A |
| IL-10 | Down-regulates Th1 cytokines, enhances B cell survival and antibody production | 18 | Plasma | Systemic Inflammation | Bioplex assay3 (Biorad) | N/A |
| TNFα | Secreted by macrophages, regulates cell proliferation, apoptosis and differentiation | 18 | Plasma | Systemic inflammation | Bioplex assay3 (Biorad) | N/A |
| MIP1β | Inflammatory cytokine produced by monocytes, chemotactic (CCL4) | 18 | Plasma | Systemic inflammation | Bioplex assay3 (Biorad) | N/A |
| Ferritin (serum) | Acute phase protein, involved in iron storage; increased by IL-1b and TNFa | 6 & 18 | Plasma | Systemic inflammation | ELISA (Immunodiagnostic Systems Ltd) | |  | | --- | | 232.89150.89  45.6754.28  ng/ml | |
| C Reactive Protein (CRP) | Acute phase protein produced by the liver, interacts with immune effector systems | 6 & 18 | Plasma | Systemic inflammation | ELISA (Immundiagnostik AG) | 1.073.62  3.017.70  mg/l |
| sCD14 | Soluble CD14; shed by activated monocytes, binds LPS | 6 & 18 | Plasma | Systemic inflammation | ELISA  (R&D Systems) | 1691.41645.08  1965.57704.22 |
| Endocab | Antibody produced against bacterial lipopolysaccharide | 6 & 18 | Plasma | Systemic inflammation | ELISA  (Hycult Biotech) | 28.8540.68  10.6737.62  gmu/ml |
| Activin | Growth regulation factor; associated with muscle wasting in cachexia, log transformed | 6 | Plasma | Systemic inflammation | ELISA  (R&D Systems) | 6.461.13 |
| Vitamin D | Absorption of calcium, iron, magnesium, phosphate and zinc | 6 & 18 | Plasma | Nutritional | ELISA  (Quantikine) | 35.1617.87  61.3323.96  nmol/l |
| Zinc | Essential mineral, deficiency leads to growth inhibition and immune deficiency | 6 & 18 | Plasma | Nutritional | Atomic absorption spectrophotometry | 726.16110.61  771.69146.91  ug/l |
| Retinol binding protein | Transport protein for vitamin A | 6 & 18 | Plasma | Nutritional | ELISA  (Quantikine) | |  | | --- | | 25197.691256.56  29736.9815137.97  ng/ml | |
| HAZ | Height-for-age Z score, stunting | 18 | Clinic measurement4 | Nutritional | N/A | -0.841.06 |
| WAZ | Weight-for-age Z score, underweight | 18 | Clinic measurement4 | Nutritional | N/A | -1.030.92 |
| WHZ | Weight-for-height Z score, wasting | 18 | Clinic measurement4 | Nutritional | N/A | -0.131.04 |
| Exclusive breastfeeding | Days when breast milk was the only food taken up to 18 weeks of age | 186 | Surveillance | Nutritional | N/A | 91.1143.40 |
| Expenditure | Monthly expenditure for household | n/a | Questionnaire5 | Maternal health & SES | N/A | 11504.747241.52 |
| Income | Monthly income for household | n/a | Questionnaire5 | Maternal health & SES | N/A | 12762.399409.93 |
| Maternal education | Education considered a “yes” if primary school was attended | n/a | Questionnaire5 | Maternal health & SES | N/A | 71.14% |
| Maternal height | Centimeters | n/a | Questionnaire5 | Maternal health & SES | N/A | |  | | --- | | 150.335.52 | |
| Maternal weight | kilograms | n/a | Questionnaire5 | Maternal health & SES | N/A | 49.319.41 |
| Treated water | Access to treated water | n/a | Questionnaire5 | Sanitation | N/A | 60% |
| Septic tank/toilet | Access to toilet with a septic tank | n/a | Questionnaire5 | Sanitation | N/A | 52.43% |
| No shared toilet | Access to private toilet not shared with neighbors | n/a | Questionnaire5 | Sanitation | N/A | 14.71% |
| No open drain | Covered drain near home | n/a | Questionnaire5 | Sanitation | N/A | 59% |

1A lactulose/mannitol solution containing 250 mg/mL of lactulose and 50 mg/mL of mannitol. Children were given the solution at weeks 12, 24, 40 and 104 at 2 mL/kg of weight up to 20 mLs. Urine was collected for 2 hours following the lactulose/mannitol dose using pediatric urine collection bags

2 Diarrheal surveillance: diarrheal burden was captured as described previously (11).

3 Custom 8-plex assays were designed. Cytokine data were categorised into bottom 50th percentile, 50-75th percentile, and 75-100th percentile, due to a high percentage of out of range values (30-50%). Subsequent analyses report association of 50-75th percentile as (2), and 75-100th percentile as (3). Out of range values were imputed with the lowest measured value for each plate. All other biomarkers are analysed continuously with no percentile grouping.

4 Duplicate anthropometry measurements were taken at every clinic visit using a calibrated digital scale and standardised supine length measurement and averaged to produce a final measurement. Measurements were converted to standardised z-scores for creation of WAZ, HAZ, and WHZ scores7. Malnourished children 3 or more SD below the mean were treated or referred to nutrition centers.

5 Field research assistants consented and enrolled mothers, after which a socio-economic questionnaire was administered as described previously (11). Maternal and infant height and weight were also measured.

6Measured as days of incidence up to 18 weeks of age

Supplemental Table 2: Measurement of vaccine response.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Vaccine1** | **Age of response measurement** | **Protective cut-off value** | **Range of responses** | **% below protection cut-off value** | **MeanS.E** |
| OPV12 | 18 | Log2=3 | 2.5 – 10.5 | 11.2 | 9.012.51 |
| OPV22 | 18 | Log2=3 | 2.5 – 10.5 | 3.4 | 9.871.36 |
| OPV32 | 18 | Log2=3 | 2.5 – 10.5 | 11.2 | 8.282.56 |
| Rotarix® IgA3 | 18 | 20 U/ml | -9.71 – 20436 6 | 68.5 | N/A |
| Rotarix® Success4 | 18 – 52 | Any diarrhea with rotavirus antigen | N/A | 14.0 | N/A |
| Tetanus 18 weeks5 | 18 | 0.1 IU/ml | 0.21 – 16.03 | 0 | 2.782.01 |
| Tetanus 52 weeks5 | 52 | 0.1 IU/ml | -2.09 – 1.05 | 0.17 | -0.420.47 |
| *H. influenzae* B5 | 52 | 0.15 μg/ml | -2.00 – 1.80 | 8.97 | 0.190.72 |
| Diptheria5 | 52 | 0.1 IU/ml | -2.74 – 0.08 | 7.92 | -1.270.49 |
| Pertussis-Ptx5 | 52 | 20 EU/ml | 0.00 – 3.00 | 76.9 | 0.770.65 |
| Measles5 | 52 | 0.12 IU/ml | -2.18 – 1.28 | 3.79 | -0.130.54 |

1 Vaccine responses are log10 transformed. The national Bangladesh EPI schedule includes BCG, pentavalent vaccine (diphtheria, pertussis, tetanus, *Hemophilus influenzae* B, hepatitis B), bivalent measles-rubella, monovalent measles, and trivalent OPV. Trivalent OPV and pentavalent vaccine were administered at 6, 10, and 14 weeks of age. Rotavirus vaccine (ROTARIX®) is not part of the EPI, and was delivered to half the enrolled children to determine efficacy as a secondary objective of the PROVIDE study. ROTARIX® was administered at 10 and 17 weeks of age in one half of the children, and response was measured at 18 weeks of age

2Poliovirus Sabin types 1, 2, and 3 serum neutralising antibody assays were performed at the Centers for Disease Control and Prevention (Atlanta, Georgia), using WHO-standardised assays.

3 Rotavirus IgA was measured in 18 week plasma with an enzyme-linked immunoassay as described (11,12)

4All diarrheal stool samples were tested for rotavirus antigen by ELISA (ProSpecT, Oxoid ltd., Hampshire, UK)6, For the 350 children who received Rotarix, 14% had diarrhoea that was positive for rotavirus fecal antigen between 18 and 52 weeks.

5 IgG response to intramuscular vaccines tetanus, measles, pertussis-Ptx, diphtheria, *Haemophilus influenzae* b was measured with luminex bead assay as described (33).

6Cut-off set at 20 U/ml for analysis