**Standardization of analyte measures**

Quality Control Samples

A total of 40 quality control samples from the PEL site were sent to each study laboratory, as well as to an external LC-MSMS laboratory (the Mayo Clinic, Rochester, MN). These included: 27 unique samples collected after L:M dosing, 3 unique urine samples collected during a 5-hour urine collection prior to which no L:M had been administered, and 10 duplicate samples. Details of the Mayo Clinic LC-MSMS protocol have previously been reported.38

In addition to these samples, for three of the four laboratories (the University of Ceara, icddr,b and Oregon Analytics), additional groups of samples was run by both the site laboratory and by the Mayo clinic. These include:

University of Ceara Laboratory–

In addition to the 27 QC samples described above, an additional seventy-three (73) samples from the Peruvian MAL-ED cohort, and one hundred (100) samples from the BRF MAL-ED cohort and from the nested MAL-ED case-control, were tested by both the University of Ceara and by the Mayo clinic.

Oregon Analytics-

The same 73 samples mentioned above were also tested by the Oregon Analytics lab, allowing for comparison of LC-MSMS to LC-MSMS.38

Icddr,b -

Results from one hundred and twenty seven (127) samples from the PROVIDE study,52 that had been measured on both the ICDDR,B HPLC-PAD system and by the Mayo Clinic, were shared with MAL-ED for the purpose of making comparisons between samples.. The laboratory protocols for determination of the urinary concentration of the probes were also identical.

Statistical Methods

 Standardization of results between study laboratories

For each of the quality control samples described above, lactulose concentration, mannitol concentration, and the L:M Recovery ratio were compared between each platform and the HPLC gold standard. Two sided t-tests were used to test whether the mean concentration of these QC samples varied between what was reported by the study laboratories, and what was reported the gold standard (Mayo Clinic) laboratory.

Adjustment factors were calculated as the geometric mean lactulose or mannitol concentration of the QC samples as determined by the Mayo Clinic laboratory, divided by the geometric mean concentration of the QC samples as determined by each of the site laboratories. Similarly, the adjustment factor for the L:M ratio was calculated as the quotient of the geometric mean L:M ratios (rather than the ratio of the corrected concentrations). The exception to this was the icddr,b laboratory, for which the adjustment factors were calculated based on PROVIDE rather than MAL-ED QC data.

For all L:M results from each site, “adjusted” urinary lactulose and mannitol concentrations the LM ratio were then taken to be the original concentration multiplied by the adjustment factor associated with that laboratory. Adjusted L:M ratios were calculated as the original L:M ratio multiplied by their adjustment factor for that laboratory

Appendix 2: Sample L:M Z-score Calculations

Example 1: A 3-month old girl with an adjusted % mannitol recovery of 7.8395 would have a box-cox transformed value of (7.8395^0.377 - 1)/0.377=3.11, and 3.11 minus the mean and divided by the standard deviation of the Brazil distribution (3.11-0.22)/1.39, would give a z-score of 2.06.

Example 2: A 6-month old boy with an adjusted % lactulose recovery of 0.8485 would have a box-cox transformed value of (0.8485^0.048) -1/0.048=-0.16, and -0.16 minus the mean and divided by the standard deviation of the Brazil distribution (-0.16-(-1.82))/0.74 would give a z-score of 2.24.

Example 3: A 9-month old girl with an L:M ratio of 0.41 would have a box-cox transformed value of (0.41^(-0.262)-1)/(-0.262)= -1.004, which minus the mean and divided by the sd of the Brazil distribution (-1.004-(-4.02))/1.95 would give a Z score of 1.55.