

APPENDIX I

Definition of Indices of Socioeconomic Status (SES) of Patients Participating in Study.

Persons who had tertiary education, earn more than 200 US\$ per month, resides in urban settlement and works in civil services were considered upper SES, while those that had had tertiary education, earn between 100 - 200 US\$ per month, resides in rural settlement and employed through business were considered middle SES, and persons who had at most high school education, earn less than 100 US\$ per month, resides in rural settlement and works as peasant farmer or unemployed were considered lower SES.

APPENDIX II

Modified Ziehl Neelsen Stain Microscopy:

Detection of *Cryptosporidium* oocysts in the concentrated stool was done using the modified cold Ziehl Neelsen staining technique, as previously described.¹¹ Briefly, a concentrated smear of the stool was made on a clean grease-free slide and fixed in methanol for 3 min. The slide was immersed in cold Carbol fuchsin and stained for 15 min. It was then thoroughly rinsed in tap water and decolorized in 1% HCl (v/v) in methanol for 10 to 15 min. After rinsing again in tap water, the slide was counterstained with 0.4% malachite green for 30 s. The slide was then air-dried and observed under the compound light microscope using 40× objective lens for the presence of *Cryptosporidium* oocysts. Higher-magnification objectives (100× objective) was used to confirm the presence of *Cryptosporidium* spp., oocysts as small pink to red spherules on pale green background.

APPENDIX III

Determination of Fecal *Cryptosporidium* Antigen by Sandwich ELISA

This was done using Para-Tech® ELISA kit from *Medical Chemical Corp, California, USA*.

This ELISA is an in vitro immunoassay for the qualitative determination of *Cryptosporidium* species antigen in human feces. The assay uses rabbit anti-*Cryptosporidium* polyclonal antibodies to capture the species antigen from the stool supernatant. A second set of goat anti-*Cryptosporidium* polyclonal antibodies were then added which sandwiches the captured species antigen. This reaction was visualized by the addition of anti-goat polyclonal antibodies conjugated to peroxidase and the chromogen tetramethylbenzidine (TMB). The resulting blue color development was converted to an easily read yellow color by addition of an acidic "stop solution" to end the reaction. The presence of yellow color above 0.15 Optical Density of absorbance indicates presence of *Cryptosporidium* oocysts or species antigens. This procedure has Sensitivity of 100% (95% CI: 86% - 100%) and Specificity of 97% (95% CI: 93% to 99%). *Cryptosporidiosis* was defined by positive ELISA result.