

Statistical Analysis Plan

For both count and continuous primary and secondary outcomes, summary statistics including number of participants, mean, median, standard deviation, first quartile, third quartile, minimum, and maximum will be obtained for each available time interval and for each group. For categorical outcomes, frequencies and percentages will be presented. The difference in the number of incidences of URTI, duration days of URTI, missed school days, well days, illness days between Bacillus coagulans SNZ 1969 and Placebo groups will be assessed by computing the rate ratio with 95% confidence interval using a generalized linear model with log link function (Poisson or negative binomial regression model). The difference in the number of incidences of Additional Respiratory Symptoms and the duration days of Additional Respiratory Symptoms between Bacillus coagulans SNZ 1969 and Placebo groups will be assessed by computing the rate ratio with 95% confidence interval using a generalized linear model with log link function (Poisson or negative binomial regression model). The difference in the number of incidences of GITI symptoms, duration days of GITI symptoms, missed school days, well days, illness days between Bacillus coagulans SNZ 1969 and Placebo groups will be assessed by computing the rate ratio with 95% confidence interval using a generalized linear model with log link function (Poisson or negative binomial regression model).

The difference in the AUC for daily total CARIFS scores, the mean total severity scores of URTI symptoms, the AUC for daily total Additional Respiratory Tract Symptoms questionnaire scores, the AUC of GITI symptom scores, and mean of GITI symptom scores between Bacillus coagulans SNZ 1969 and Placebo groups will be assessed by two-sample t-test if the outcome is normally distributed and Wilcoxon's rank sum test if the outcome is not normally distributed. Normality assumption will be assessed using quantile quantile (Q-Q) plots. Fisher's exact test will be used to assess the difference of proportion of participants who used prescription and non-prescription cold/flu medications to treat URTI/GITI symptoms between Bacillus coagulans SNZ 1969 and Placebo. 95% confidence interval of odds ratio will be calculated. Differences between groups for sIgA, IgA, IgG, IgE, IgM, and immune response biomarkers at day 84 will be assessed by ANCOVA model adjusting for baseline values. To assess the goodness of fit of the log link models, the Poisson regression model and the negative binomial regression model will be applied to the same dataset and then the Likelihood Ratio Test will be performed. If the p-value of the test is less than 0.05, then we will use the negative binomial model as it offers a better fit of the count data. To evaluate the adequacy of ANCOVA model, diagnostics including Q-Q plots (for assessing the normality of error terms) and plots of residuals versus fitted values (to examine if the residuals are independent and have constant variance) will be generated. If these diagnostic plots indicate deviations from the model's assumptions, ranks or other transformation of the dependent variable, will be implemented. Two-sided probabilities ≤ 0.05 will be considered statistically significant. All statistical analysis will be completed using the R Statistical Software Package Version 4.3.2 or newer for Microsoft Windows.