Supplemental Digital Content 2. Methods

Co-infection, defined as an infection with both influenza viruses and other respiratory viruses at any time point during the first 9 days post-enrollment, was monitored using nasal or throat swab samples assayed by singleplex RT-PCR for 20 respiratory viruses, including influenza viruses, and bacteria (Table, Supplemental Digital Content 4).

All nasal/throat swab samples were extracted in the designated pre-amplification space using an automated Qiacube robotic platform. The extracted ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) was then tested by a singleplex One-Step Quantitative Real-Time Polymerase Chain Reaction (one-step qRT-PCR). In one-step qRT-PCR, reverse transcription and PCR amplification occurred in the same tube. Gene-specific primers were used for generating the complementary DNA (cDNA) and for subsequent cDNA amplification. During the reverse transcription phase, the RNA was converted into cDNA by reverse transcriptase enzyme. Taq polymerase then amplified the target sequence from the cDNA to a PCR product. The PCR set-up and addition of RNA were done in predesignated areas. The amplified products were never moved into pre-amplification areas. Forty-five samples were tested in each run of the singleplex RT-PCR test along with positive, negative, and no-template controls. A housekeeping gene, RNase P, was used as an internal control to determine the quality of the samples. Samples with cycle threshold values of ≥37 were re-extracted and retested. PCR data were analyzed, quality checked, and exported electronically to the laboratory database, StoneBase. Quality checks were also performed after data transfer upload to ensure the data were uploaded correctly.

Fever recurrence after Day 4 (post hoc endpoint) was defined as children who had resolution of fever up to 72 hours after treatment (Day 4) and recurrence of fever after Day 4. For influenza A and B, the analysis population for fever recurrence was the ITTI population with paired sequence data and the ITTI population, respectively.