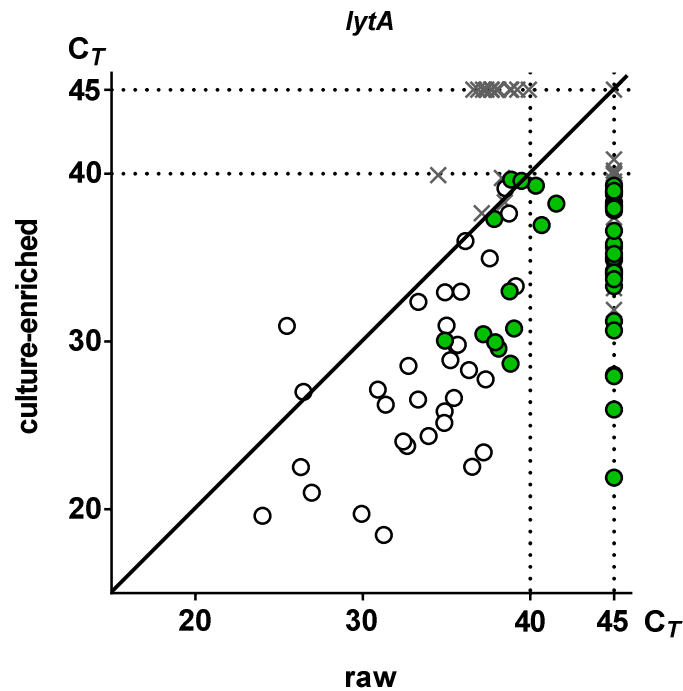


Figure S1. Impact of culture-enrichment on *S. pneumoniae* gene *lytA* detection by qPCR in saliva samples (n=270) from elderly.



Each dot or cross represents an individual sample. The position of symbols corresponds to C_T values for *lytA*-specific signals in DNA extracted from raw and culture-enriched sample of saliva as marked on corresponding axes. Dots represent 76 saliva samples classified as positive and crosses represent 194 saliva samples classified as negative for *S. pneumoniae* in the study. Open dots represent 32 saliva samples classified as positive for *S. pneumoniae* in both raw and culture-enriched samples. Green dots represent 44 samples classified as positive only after culture-enrichment. Dotted lines mark the threshold assigned to discriminate between positive ($C_T < 40$) and negative samples, and the total number of 45 cycles in qPCR reaction. There was a significantly higher number of saliva samples classified as positive for *S. pneumoniae* after culture-enrichment compared to raw saliva samples (76 or 29% versus 31 or 11%; Fisher's exact, $p < 0.001$). Culture-enrichment increased the signal strength of the genes targeted with qPCR, with an average overall increase of 2.10 C_T for the *lytA* gene (maximum observed increase of 23.11 C_T) and of overall increase of 1.90 C_T for the *piaA* (maximum increase of 21.30 C_T). In the subset of 76 saliva samples identified as positive either in raw or culture-enriched sample an average increase for *lytA* was 6.92 C_T and for *piaA* was 6.64 C_T .