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.