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Suitable reference genes determination for real-time PCR using induced sputum samples

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The best reference genes to use for normalisation when performing reverse transcriptase qPCR from sputum cells were assessed http://bit.ly/2knSDXm

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ABSTRACT Induced sputum is a non-invasive method of collecting cells from airways. Gene expression analysis from sputum cells has been used to understand the underlying mechanisms of airway diseases such as asthma or chronic obstructive pulmonary disease (COPD). Suitable reference genes for normalisation of target mRNA levels between sputum samples have not been defined so far.

The current study assessed the expression stability of nine common reference genes in sputum samples from 14 healthy volunteers, 12 asthmatics and 12 COPD patients.

Using three different algorithms (geNorm, NormFinder and BestKeeper), we identified HPRT1 and GNB2L1 as the most optimal reference genes to use for normalisation of quantitative reverse transcriptase (RT) PCR data from sputum cells. The higher expression stability of HPRT1 and GNB2L1 were confirmed in a validation set of patients including nine healthy controls, five COPD patients and five asthmatic patients. In this group, the RNA extraction and RT-PCR methods differed, which attested that these genes remained the most reliable whatever the method used to extract the RNA, generate complementary DNA or amplify it.

Finally, an example of relative quantification of gene expression linked to eosinophils or neutrophils provided more accurate results after normalisation with the reference genes identified as the most stable compared to the least stable and confirmed our findings.