Methodology on line supplement

Patient recruitment

All patients had COPD as defined by a forced expiratory volume in one second (FEV_1) of $\leq$ 80% and FEV_1 to forced vital capacity (FVC) ratio below 70% with β_2 agonist reversibility of less than 15% or 200ml. Patients were excluded if they had other significant respiratory diseases. Patients were recruited when stable, with no exacerbations reported in the preceding month.

Sample processing; upper and lower airway

Patients were instructed to blow their nose prior to the swab being passed gently through the nose towards the posterior nasopharynx. The swab was rotated 5-6 times and allowed to remain in place for 5 seconds. The swab was then immediately placed in an eppendorf containing 0.5ml PBS (phosphate buffered saline) and stored at -80°C until RNA extraction.

Sputum samples were examined as soon as possible and within two hours of collection. The sample was separated from contaminating saliva and processed using previously published methods (15). The sputum was mixed with nine times its weight of PBS and agitated with siliconised glass beads for 10 minutes. 0.5ml aliquots were stored at -80°C.

RNA extraction, reverse transcription

RNA was extracted from NPS using the High Pure Viral RNA kit (Roche) according to manufacturer instructions. HRV 16 (gift from Virology, St. Bartholomew's hospital UK), was also isolated using this method. RNA was extracted from sputum using Tri-

reagent LS (Sigma) according to the manufacturer instructions. Pellets were resuspended in 30µl RNase free water.

cDNA was prepared using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) following the manufacturer instructions. 10µl of extracted RNA was used per reverse transcription reaction. Positive (virus spiked samples), negative (water) and a template control were used for each run.

The primers and probes were designed in our lab; forward primer; 5'-TGADTCCTCCGGCCCCT-3' and reverse primer; 5'-AAAGTAGTYGGTCCCRTCC-3'. The probe was a minor groove binding probe; 6-FAM-AATGYGGCTAACCT-MGB.

PCR

Real-time PCR was performed using the ABI Prism 7500 Real Time PCR System (Applied Biosystems). 25µl reaction volumes were set up (12.5µl QuantiTect Probe PCR Master Mix (ROX reference dye, Qiagen), 1µl forward and reverse primers (20µM), 0.35µl probe (20µM), 2.5µl template and 7.65µl RNase free water. PCR conditions: 95°C 15 min, 40 cycles of 95°C 15 sec and 58°C 80 sec. Samples were initially run as singles and any positive samples were repeated in duplicates and data pooled. If the two runs varied, the sample was re-run in triplicate.

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Controls and standards for real-time RT PCR

At the RNA extraction stage at least 1 positive and 1 negative control were included per 16 samples. Further template controls were included at the RT and PCR amplification stage. Standards were prepared as follows: HeLa-Ohio cell monolayers at 90% confluence were infected with HRV 16 at 1 moi and incubated at 33°C, 5% CO_2 on a shaking platform in the presence of 2mM $MgCl_2$ for 24 hours until CPE was observed. The supernatant was aliquoted and stored at -80°C. Virus was titrated by plaque assay (16) with 2mM $MgCl_2$ added to the overlay and used as standards for real-time RT-PCR.

As an additional standard, a 137 bp region of the 5'UTR HRV containing the amplicon of the real-time RT-PCR was amplified, cloned into - pCRII-TOPO vector and transformed into either TOPO10 or Mach1 bacterial strain (Invitrogen). Clones were PCR verified and sequenced. Purified plasmids (Qiaprep Miniprep spin kit, Qiagen) were linearised with BamHI, DNAse treated, phenol chloroform extracted and *in vitro* transcribed using the Riboprobe *in vitro* transcription system (Promega). Samples were again phenol chloroform extracted and the size of the *in-vitro* transcript verified on a denaturing RNA agarose gel. Aliquots of the *in-vitro* transcripts were frozen at -80^{0}C prior to use as a standard for qRT-PCR.

A standard curve prepared from tissue culture grown virus extracted in the same manner as the clinical samples was set up at the RT stage using 6 10-fold dilutions. A second separate standard curve was included at the PCR stage. Results were only accepted if both standard curves were comparable, had an r^2>0.98 and a slope between -3.1 to -3.6. In addition a reverse transcribed standard curve of the RNA

transcript was included in duplicate on the quantification plates. A cycle threshold (ct)-value of 38 was taken as a cut off for positivity (1 pfu/ml) for sputum and for NPS a ct value of 39.5 (1 pfu/ml) was used.