# Quantitative detection of *Streptococcus pneumoniae* in nasopharyngeal fluid using droplet digital PCR method

EP0941
**27th ECCMID**
22 – 25 April 2017,
Vienna, Austria.

Alexey Mokhov[1], Julia V Sopova[2], Irina Tsvetkova[1], Vladimir Gostev[1], Marina Wolkowa[1], Olga Kalinogorskaya[1], Dmitry Kolesnik[3], Artemiy Goncharov[4], Sergey Sidorenko[1]
[1.] Scientific Research Institute of Children's Infections, Saint Petersburg, Russia
[2.] St.Petersburg State University, Saint Petersburg, Russia
[3.] Regional office of Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing, Saint Petersburg, Russia
[4] North-Western State Medical University named after I.I. Mechnikov, Saint Petersburg, Russia

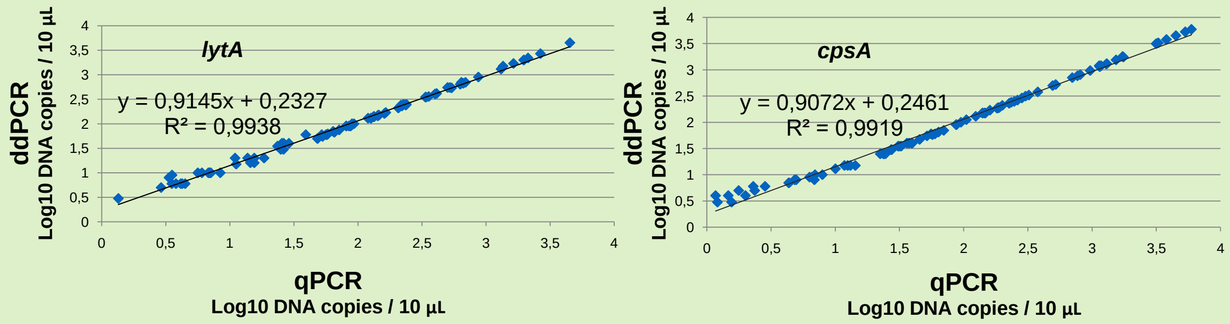
Professor Popov Str. 9
Saint Petersburg
197022, Russia
sidorserg@gmail.com

**Background:** The droplet digital PCR (ddPCR) is a method of absolute nucleic acid quantification based on the partitioning of individual analyte molecules into many replicate reactions at limiting dilution one or zero molecules in most reactions (Hindson MC, 2013). Here we present data of quantitative detection of *S. pneumoniae* DNA in clinical samples using ddPCR.

**Material/methods:** The study was approved by local ethics committee. The nasopharyngeal fluids were sampled from healthy children and pediatric patients with acute respiratory infections using eSwab system (Copan, Italy). DNA was extracted with DNA–SORB B kit (ILS, Russia). Two target genes were used for DNA screening – *lytA* (pneumococcal autolysin) and *cpsA* (Wzy-polymerase). The primers and probes sequences were designed accordingly CDC recommendations. Droplet digital PCR QX100 system (Bio-Rad, USA) with standards kits were used in the study. Quantitative real-time PCR (qPCR) was used for comparison of data (CFX96, Bio-Rad, USA). The standard samples were made using *lytA* and *cpsA* gene fragments which were cloned into the plasmid, using pJET1.2 AR kit (Thermo Fisher Scientific, Waltham, MA). The linear calibration curve was performed with standard sample with 10-fold dilutions, from $10^1$ to $10^6$ DNA copies/10 µL. The inter-assay variation coefficients (*CVs)* for pneumococcal DNA standards were determined in the 9 independent runs (Table 2).

**Results**: 234 samples from healthy children were tested using ddPCR and qPCR. Pneumococcal DNA was detected in 37 cases (15.8%) by both methods, discrepancies between methods were not found. Fifty samples, positive for pneumococcal DNA, were additionally included in this study for comparison of the methods. Overall, the values of DNA copies of *lytA* and *cpsA* genes were in range 1–6000 DNA copies/10 µL. Positive correlation was observed between two methods (Figure 1).

**Figure 1. Correlation of Quantitative real-time PCR and ddPCR methods**




The analytical sensitivity of qPCR and ddPCR methods was equaled 1 and 3 DNA copies/10 µL, correspondingly. The CVs for *lytA*- and *cpsA* DNA measuring in the samples were determined for the subranges: 0–100, 100–1000 and 1000–6000 DNA copies/10 µL (Table 2).
The differences of logarithmic medians for *lytA* and *cpsA* DNA copies per 100 µl of sample (the transport medium of eSwab system) were significant for healthy children and pediatric patients ($p<0.01$) (Figure 2).

**Figure 2. *lytA* and *cpsA* DNA copies**

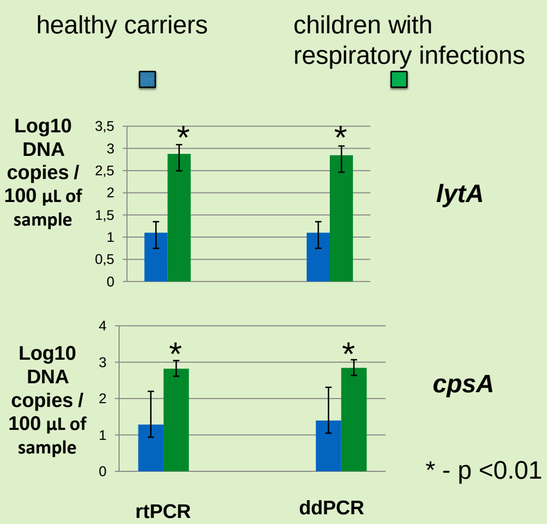



**Table 2. The methods reproducibility and analytical sensitivity**

| | Quant. real-time PCR | dd-PCR |
|---|---|---|
| **lytA-standards** | **Inter-assay CV (%)** | |
| 14 copies/ 10 µl | 4,20 | 3,50 |
| 140 copies/ 10 µl | 0,50 | 0,50 |
| 1400 copies/ 10 µl | 0,10 | 0,10 |
| **cpsA-standards** | | |
| 38 copies/ 10 µl | 4,30 | 6,00 |
| 378 copies/ 10 µl | 0,30 | 0,30 |
| 3780 copies/ 10 µl | 0,02 | 0,02 |

**Conclusion:** These results show a high sensitivity and good reproducibility of quantitative detection of pneumococcal DNA with ddPCR. Concerning the fact, that there is no need to use the standard curve calibration with ddPCR, this method can have the advantages for the pathogens DNA quantitative detection in clinical specimens. According to received data, ddPCR can be used in diagnostic procedures for detection *S. pneumoniae*.