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Quantitative detection of Streptococcus pneumoniae in nasopharyngeal swabs using droplet digital PCR method

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Background: 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, resulting in one or zero molecules in most reactions (Hindson MC, 2013). Detection of pneumococcal DNA in clinical samples requires a high sensitive and specificity. Here we present data of quantitative detection of *S.pneumoniae* in clinical samples using ddPCR.

Material/methods: Study was approved by local ethics committee. Nasopharyngeal swabs were sampled from healthy children (from 1 to 5 years) using eSwab system (Copan, Italy). DNA was extracted with DNA–SORB-B kit (ILS, Russia), during 24 hours after specimens were sampled. Two target genes were used for DNA screening – *lytA* (pneumococcal autolysin) and *cpsA* (Wzy-polymerase). The primers and probes (Taqman) sequences were designed accordingly CDC

recommendations. Droplet digital PCR QX100 system (Bio-Rad, USA) with standards kits were used in the study. Real-time quantitative PCR (qPCR) was used (CFX96, Bio-Rad, USA) for comparison. Standard samples were made using *lytA* and *cpsA* gene fragments which were cloned into the plasmid, using kit pJET1.2 AR (Thermo Fisher Scientific, Waltham, MA). Linear calibration curve was performed with standard sample with 10-fold dilutions, from 10^1 to 10^6 DNA copies/10 µL.

Results: Overall, 234 samples were included in the study. Among these, 37 (15.8%) samples were *S.pneumoniae* positive (*lytA*+, *cpsA*+). The amount of *lytA* and *cpsA* copies were in range 1–4500 copies/10 µL. Positive correlation was observed between two methods (Figure 1A/1B). Linear correlation coefficients (R^2) for two methods were the same for *lytA* and *cpsA*: 0.99 and 0.97, respectively. But the most differences between measurements (qPCR vs ddPCR) were in low-copy area, in range 0–100 copies/10 µL. For instance, copy number variations for *lytA* were 28.6%, 0.2% and 0.08% in subranges 0–100, 100–1000 and 1000–4500 copies/10 µL, respectively. Copy number variations for *cpsA* were 11%, 0.4% and 5.1% for the same subranges 0–100, 100–1000 and 1000–4500 DNA copies/10 µL. Inter-assay CVs for *lytA* for three individual standards (14, 140 and 1400 DNA copies/10 µL) were 4.2%, 0.5% and 0.1% for measuring with qPCR and 3.5%, 0.5% and 0.1% for measuring with ddPCR. Inter-assay CVs for *cpsA* for three individual standards (38, 378 and 3780 DNA copies/10 µL) were 4.3%, 0.3% and 0.02% for measuring with qPCR and 6%, 0.3% and 0.02% for measuring with ddPCR.

Conclusions: These results show high sensitivity and good reproducibility of quantitative detection of pneumococcal DNA with ddPCR. There is no need to use the standard curve calibration and this method works best in low-copies area, this is the main advantage of the ddPCR. The ddPCR is useful molecular tool in diagnostic procedures for detection *S.pneumoniae*.

Figure 1. Quantitative real-time PCR and ddPCR methods correlations