Comparison of the sensitivity of six PCR formats for the detection of *P. aeruginosa* in sputum

P. Deschagt1, T. De Baere1, L. Van Simaeys1, S. Roels1, P. Schelstraete2, S. Van Daele3, F. De Baets3, M. Vanecehouette1, 1Laboratory Bacteriology Research (LBR), University Ghent, Ghent, Belgium; 2Dept. Otolaryngol., Rigshospitalet, Copenhagen, Denmark; 3Cystic Fibrosis Center, Ghent University Hospital, Ghent, Belgium

**Introduction:** Colonization of the airways of CF patients with *Pseudomonas aeruginosa* results in faster decline of the lung function and decreased life expectancy. Detection of infection with this pathogen as early as possible enables to start the eradication treatment as soon as possible.

**Aim:** To compare the sensitivity of six PCR formats for a dilution series of *P. aeruginosa* in sputum.

**Materials and Methods:** Sputa from CF patients positive for *P. aeruginosa* were liquefied by adding v/v Sputasol and were 1/5 serially diluted in a pool of liquefied sputa from CF patients negative for *P. aeruginosa*. DNA was extracted with the bioMérieux easyMAG Nuclisens extractor. Conventional PCR was performed using the Veriti 96-Well Thermal Cycler, with oprL as the target, followed by capillary resp. agarose gel electrophoresis. Different real-time PCR formats on the LightCycler 1.5 were used: SYBR Green I, HybProbes and TaqMan probe, were compared with the TaqMan® *P. aeruginosa* detection kit on the ABI 7000.

**Results:** For conventional PCR, *P. aeruginosa* could be detected up to dilution 6 using agarose gel electrophoresis and up to dilution 7 with capillary electrophoresis. For real-time PCR, *P. aeruginosa* could be detected with SYBR Green 1 up to dilution 7 and up to dilution 8, i.e. up to 20–40 cfu/mL, with the HybProbes, TaqMan probe and the TaqMan® *P. aeruginosa* detection kit.

**Conclusion:** Real-time PCR, using the HybProbes, the TaqMan probe or the TaqMan® *P. aeruginosa* detection kit is 5–25 fold more sensitive for the detection of *P. aeruginosa* than conventional PCR and real-time PCR using SYBR Green 1.