

O546

Abstract (oral session)

**High-throughput MLST - bringing molecular typing to the next level**

S.A. Boers\*, W.A. van der Reijden, R. Jansen (Haarlem, NL)

**Introduction:** MultiLocus Sequence Typing (MLST) is a widely used system for typing microorganisms by sequence analysis of seven housekeeping genes. MLST protocols are published for many species and online databases have proven to be a powerful resource in studying the (global) epidemiology. The main advantage of MLST compared to other typing techniques is the unambiguity and transferability of sequence data. The main disadvantage is the high costs to generate the sequence data. **Objective:** Here we introduce the High Throughput MLST (HiMLST) method that employs Next Generation Sequencing (NGS), which delivers large quantities of high quality MLST data at low prices. **Methods:** The HiMLST protocol consists of two steps. In the first step the seven MLST targets are amplified by PCR in multi-well plates. During this PCR the amplicons of each strain are provided with a unique DNA tag, the Multiplex Identifier (MID). In the second step all amplicons are pooled and sequenced in a single NGS run (GS Junior, Roche). After the sequencing run, the MLST profile of each individual strain can be easily generated using its unique MID. **Results:** With the HiMLST we have generated 96 MLST profiles in a single run. The HiMLST was employed for *Legionella pneumophila*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus pneumoniae* and will be applicable to many other species. Moreover, the use of MIDs allows the combined sequencing of different species in a single NGS run. Currently, the HiMLST reduces the cost of MLST by a factor 10 compared to traditional methods. It is expected that the costs can be reduced further by introducing low volume PCRs and automated processing of reagents and sequence data. In this way, the HiMLST capacity can be doubled while retaining the high quality of the sequences. **Conclusion:** The introduction of HiMLST paves the way for a broad employment of the MLST as a high quality and cost effective method for typing microbial species.