Raman spectroscopy in clinical microbiology
recent steps to realize diagnostic applicability

Kees Maquelin\(^1\), Diana Willemse-Erix\(^1\), Maarten J. Scholtes\(^1\), Patricia C.A.M. Buijtels\(^2\), Alex van Belkum\(^2\) and Gerwin J. Puppels\(^1, 3\)

\(^1\) Center for Optical Diagnostics & Therapy, Dept. of General Surgery, Erasmus MC University Medical Center Rotterdam, PO Box 2040, 3000 CA Rotterdam, The Netherlands
\(^2\) Department of Medical Microbiology & Infectious Diseases, Erasmus MC University Medical Center Rotterdam, The Netherlands
\(^3\) River Diagnostics BV, PO Box 25229, 3001 HE Rotterdam, The Netherlands.

In the Center for Optical Diagnostics & Therapy we are developing Raman spectroscopy for diagnostic microbiology. Identification of \(\text{Mycobacterium}\) species remains a troublesome task in a clinical laboratory. We have analyzed the capabilities of Raman spectroscopy for this task. First, the reproducibility of Raman spectroscopy was evaluated for killed mycobacteria (by heat and formalin) versus viable mycobacteria. The spectra of the heat-inactivated bacteria showed minimal differences as compared to the spectra of viable mycobacteria. Therefore, identification of mycobacteria appears possible without biosafety level III precautions. 16S rRNA sequencing was used as a gold standard for the identification of \(\text{M. tuberculosis}\) complex strains and the most frequently found strains of nontuberculous mycobacteria (NTM). Sixty-three strains, belonging to 8 distinct species were analyzed. The sensitivity of Raman spectroscopy for the identification of \(\text{Mycobacterium}\) species was 95.2\%. All \(\text{M. tuberculosis}\) strains were correctly identified (7/7; 100\%) as were 54 of 57 NTM strains (94\%). The differentiation between \(\text{M. tuberculosis}\) and NTM was invariably correct for all strains.

Besides species identification, determination of the clonal relatedness of microbial isolates is important to detect transmission of microorganisms between patients, e.g. during a hospital outbreak. Recent observations during a study on the transmission of methicillin resistant \(\text{S. epidermidis}\) (MRSE) will be discussed that will show high correlation between genetic typing methods and Raman spectroscopy.

Finally, some results will be presented that came out of the cooperation between River Diagnostics and the Center for Optical Diagnostics & Therapy. The cooperation is focused on the development of easy-to-use methodology and instrumentation for routine operation of Raman spectroscopy in a clinical diagnostic microbiology laboratory.