Differentiation of Microorganisms by NIR-Raman Spectroscopy

K. Maquelin[1], L.-P. Choo-Smith[1], T. van Vreeswijk[1], H.Ph. Endtz[2], H.A. Bruining[1] and G.J. Puppels[1]

[1]Laboratory for Intensive Care Research and Optical Spectroscopy, Erasmus University Rotterdam & Dept. General Surgery 10M,University Hospital Rotterdam "Dijkzigt", Dr. Molewaterplein 40, 3015 GD Rotterdam, The Netherlands, Tel. ++ 31 10/ 4635980, e-mail: puppels@hlkd.azr.nl.
[2]Inst. Medical Microbiology and Infectious Diseases, University Hospital Rotterdam "Dijkzigt", Rotterdam, The Netherlands.

Routine clinical microbiological identification of pathogenic microorganisms is largely based on nutritional and biochemical tests. Laboratory results can be presented to a clinician after 2-3 days for the majority of clinically relevant microbes. A large portion of this time is required to obtain pure cultures and enough biomass for the tests to be performed. In the case of severely ill patients, the unavoidable time delay associated with such identification procedures can be fatal. A novel identification method based on confocal Raman microspectroscopy will be presented. With this method it is possible to obtain Raman spectra directly from microbial microcolonies on the solid culture medium, which have developed after only 6 hours of culturing for most commonly encountered organisms.[1]

A selection of bacterial strains belonging to clinically relevant species was tested over a longer period of time (~6 months). Prior to Raman measurements, the strains were cultured on Mueller Hinton medium (bacteria) or Sabouraud+2% dextrose medium (yeast) for 6 hours. Per strain, Raman spectra were obtained directly from 5 still on the solid culture medium. Ten 30-sec. spectra were taken at random positions from each microcolony. All data was analysed as described previously.[1] Briefly, first derivative spectra were calculated, the region from 400 to 1800 cm^{-1} was selected and spectral contributions from the culture medium and water were subtracted. Data was analysed using principal component analysis, hierarchical cluster analysis and linear discriminant analysis. Identification of different microbial species was possible using this approach. Therefore, this technique potentially enables rapid (same day) identifications, but also preserves the sample allowing it to be double-checked with traditional tests. This, combined with the speed and minimal sample handling indicate that confocal Raman microspectroscopy has much potential as a powerful new tool in clinical diagnostic microbiology.

[1]K. Maquelin, L.P. Choo-Smith, T. van Vreeswijk, H.P. Endtz, B. Smith, R. Bennett, H.A. Bruining and G.J. Puppels, 'Raman spectroscopic method for identification of clinically relevant microorganisms growing on solid culture medium', *Anal Chem*, **72,** 12-9 (2000).