Fourier-Transform Infrared Spectroscopy (FT-IR) as Tool in the Routine Diagnostics of the Microbiology Laboratory

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Introduction / objectives: Although miniaturized biochemical and immunological testing has progressed and molecular-genetic tests are evolving rapidly, time consuming conventional batteries of biochemical tests together with morphological phenotyping are still the basis for identification and differentiation of bacteria and fungi in the routine microbiological laboratory. Several (semi) automated systems based e.g. on gas-chromatography, frequency-pulsed, electron-capture, high-performance gas-liquid chromatography, nuclear magnetic resonance spectroscopy, mass spectroscopy, impedance and conductivity measurement, bioluminescence fluorescence-light scattering, flow-cytometry, direct fluorescence microscopy, infrared spectroscopy (IR) and variations or combinations thereof were developed in the past decades to improve and accelerate microbiological diagnostics of viable and non-viable micro-organisms. Because IR-fingerprinting methods significantly improved the last years and commercially manufactured IR-systems for microbiology became available, a variety of micro-organisms have been investigated by FT-IR in this period. Therefore the feasibility of FT-IR was examined for the ability of rapid and reliable identification of bacteria and fungi from human, veterinary, pharmaceutical and environmental origin.

Material and Methods: In comparison to standard microbiological identification techniques and genotyping, FT-IR fingerprinting was investigated with a great number (≥ 10, partly up to 400 strains) of isolates and selected culture collection strains of bacteria and yeasts (*Staphylococcus* spp., *Streptococcus* spp., *Listeria* spp., Nonfermenters (e.g. *Pseudomonas* spp., *Burkholderia* spp., *Acinetobacter* spp.), *Enterobacteriaceae*, *Haemophilus* spp., yellow pigmented bacteria (e.g. *Pseudomonas* spp., *Flavobacterium* spp., *Pantoea* spp., *Aureobacterium* spp. *Brevibacterium* spp., *Bacillus* spp., *Micrococcus* spp., etc.), *Candida* spp., *Cryptococcus* spp., black yeasts (e.g. *Exophiala* spp., *Malessezia* spp.), pathogenic algae (*Prothoteca* spp.), and dermatophytes (e.g. *Trichophyton* spp., *Microsporum* spp.). Bacteria were cultured with casein peptone soy peptone medium, fungi with Sabouraud-2%-glucose medium. IR spectra were recorded of freshly prepared sample between 4000 and 600 cm^{-1} using a Bruker IFS 28/B spectrophotometer; the software OPUS® 2.2 was used for spectra analysis, data processing and cluster analysis (Ward's algorithm, average linkage). Serotyping was performed with commercially available antisera. Carbohydrate assimilations and fermentation-patterns were determined by conventional biochemical and, in parallel, with commercially available micro-panel identification systems. PCR fingerprinting was performed as described in the literature.

Results and Discussion: Development of standardised micro-organism suspension prepara-tion and adjustment of inoculum density to obtain homogenous films on the sample support turned out to be most crucial for reproducible results. Similar types of culture media showed similar spectra. Medium type and growth conditions have to be maintained for uniform results. Adherence to these prerequisites, the FT-IR method compared well with other pheno-typing methods and genotyping for all the investigated micro-organism groups. The (OPUS®) software for spectra-evaluation and data presentation is lacking many automation-features and should be improved significantly for easier operation in a routine microbiology laboratory. Overall, FT-IR proves as an additional (not the only) tool for rapid, effective, reproducible and low-cost identification of bacterial and fungal isolates to the genus, species, subspecies and strain-variation level. When appropriate data bases are available, the FT-IR system outperforms all actually commercially available miniaturised identification systems with regard to performance, rapidity, costs and reliability.