



CENTRE OF BIOLOGICAL ENGINEERING  
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## Intact-cell MALDI-TOF (ICM) mass spectrometry for rapid identification and subtyping of *Burkholderia cepacia* complex bacteria

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Matrix-Assisted Laser Desorption/Ionisation Time-Of-Flight Intact Cell Mass Spectrometry (MALDI-TOF ICMS) analyses the chemical cellular composition of microorganisms providing rapid, discriminatory fingerprints for identification and subtyping of important nosocomial pathogens. The remarkable reproducibility of this technique is based on the measurement of constantly expressed and highly abundant proteins. The usually observable molecular mass range is between 2,000 and 20,000 Da, where important ribosomal proteins appear, which is an advantage because these can be easily used as biomarkers. The *Burkholderia cepacia* complex (Bcc) comprises at least 17 closely related bacterial species that have very high metabolic versatility, are ubiquitous in the environment and can cause opportunistic infections, in particular in patients with cystic fibrosis (CF). Chronic respiratory infections caused by these bacteria are, in general, characterised by low responsiveness to antibiotic therapy and rapid reduction of lung function. Epidemiological surveys of Bcc bacteria involved in respiratory infections among the Portuguese CF population under surveillance at this Pediatric and Adult CF Treatment Center of Santa Maria Hospital (HSM), in Lisbon, have been carried out by the IST laboratory, covering isolates obtained since 1995. They belong to *B. cenocepacia* (*recA* lineages IIIA and IIIB), *B. cepacia*, *B. multivorans* and *B. stabilis* species, with an exceptionally high representation of *B. cepacia*. These isolates were classified at the species level by established molecular methods, and differentiated at the strain level, based on their ribopattern/multilocus sequence typing (MLST) profiles. However, these techniques are time-consuming and expensive. In order to overcome these limitations MALDI-TOF ICMS was successfully explored as a rapid, precise, and cost-effective tool for identification and subtyping of intact Bcc bacteria. The method was tested using isolates obtained in the HSM CF Center, including clonal variants retrieved from the same CF patients during several years of chronic colonisation, previously characterized by conventional molecular biology techniques.