MALDI-ToF Mass Spectrometry

A valid alternative for microbial identification

Rapid and accurate identification of pathogenic microorganisms in areas such as pharmaceutical and medical device manufacturing are of paramount importance. Traditional identification techniques often require subcultures on selective medium, colony isolation, Gram staining or biochemical testing, which are labour intensive and time consuming.

Rapid microbiological methods (RMMs) for microbial identification have been widely adopted by the clinical microbiology environment due to their speed and accuracy resulting in reduced risk and improved patient safety. Furthermore, as RMMs generally reduce product release cycle time, labour and cost alongside supporting real-time data analysis and high throughput, it is unsurprising that these technologies are also being adopted by the pharmaceutical and medical device industries.

Matrix Assisted Light Desorption Ionisation Time-Of Flight (MALDI-ToF)

MALDI-ToF mass spectrometry, a phenotypical RMM for microbial identification, is a unique, highly accurate alternative method that facilitates cost-effective, precise and rapid data acquisition. Since its introduction by Karas and co-workers in 1987[1], MALDI-ToF mass spectrometry has been substantially developed and is now considered to be a real alternative for bacterial identification due to the provision of rapid and specific determination analogous to molecular sequencing techniques[2], with the benefit of significant time and cost savings.

The science behind MALDI-ToF

MALDI-ToF mass spectrometry measures a distinctive pattern of highly abundant ribosomal proteins characteristic for all microorganisms. This pattern creates a unique molecular fingerprint that is matched with an extensive reference library in order to identify the micro-organism down to the species level.

In brief, a single colony from a pure culture is placed on a target plate, air dried and overlaid with a matrix containing α-Cyano-4-hydroxyl-cinnamic acid. The matrix lyses cell walls of bacteria, allowing for the extraction of protein and the embedding of the separate protein molecules in dried crystal matrix. This is subsequently subjected to laser irradiation via an intense blast of UV light.

As a result, intact proteins and the matrix rapidly evaporate into the vacuum (**Figure 1**). The released ions reach the flight tube at a mass dependent speed. Proteins and ions are characterised by different masses, so the detector is reached by ions at distinct times (time of flight). The speed of reaching the detector is measured and converted into a molecular mass.

This creates a characteristic, species-specific pattern of mass spectrum, which is transformed into a peak list and subsequently compared to a reference database. The degree of similarity between the sample peak pattern and the reference entry in the database is generated based on a biostatistical algorithm.

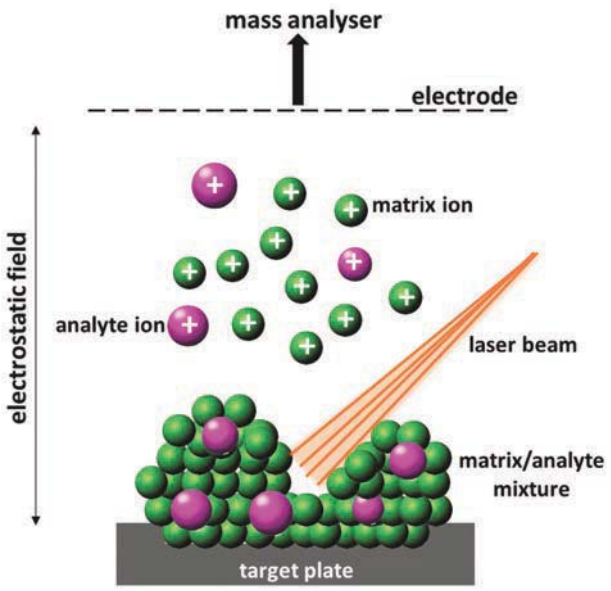

Figure 1: Principle of the MALDI-ToF bacterial identification process

Conclusion

There has been a slow but steady increase in the uptake of automated microbial identification systems by Good Manufacturing Practice accredited laboratories. MALDI-ToF mass spectrometry represents an attractive alternative to more traditional identification methods due to its accurate, precise and reliable results. Although the initial cost of implementation and validation can be high, the long-term benefits of the investment are significant.

References

1. Karas M., Bachmann D., Bahr U. & Hillenkamp F. Int. J. Mass Spectrom. Ion Processes 78, 53–68 (1987)
2. Mellmann A., Cloud J., Maier T., Keckevoet U., Ramminger I., Iwen P., Dunn J., Hall G., Wilson D., Lasala P., Kostrzewa M., Harmsen D. J Clin Microbiol 46, 1946–1954 (2008)