METHOD STATEMENT

Determinand:
Applies to all Microbiology determinands utilising a culture-based method

Matrix:
Not applicable.

Principle of Method:
Matrix Assisted Laser Desorption Ionisation Time of Flight Mass Spectrometry (MALDI-ToF MS) is an alternative confirmation method which can be applied to Microbiology methods where a sample is inoculated/cultured upon agar plates to produce discrete bacterial colonies.

Using a sterile wooden cocktail stick a small portion of a pure bacterial colony is transferred from the original isolation agar and smeared onto a 96-well stainless steel target plate. The bacteria are evenly applied across a target well to produce a thin layer.

A one micro litre aliquot of HCCA Matrix (alpha-cyano-4-hydroxy-cinnamic acid dissolved in organic solvent) is then aseptically inoculated onto the smeared well and allowed to dry at room temperature. The acidic nature of the matrix works to break down the cell-wall of the prepared bacteria, releasing the proteins contained within and trapping them within the drying matrix, which will leave behind a crystalline layer.

At this point the sample is no longer biologically active and has shown to be stable for a minimum of three days at room temperature. This enables safe transport of prepared 96-well target plates between sites if required.

The target plate is then loaded into the MALDI-ToF MS instrument. Upon commencement of an instrument run a laser will fire onto the target well causing desorption and ionisation of the bacterial proteins, which are then fired along a Time of Flight tube towards a detector. Readings from this detector undergo an automatic calculation to produce a mass-spectra of the bacterial proteins within the 2kDa to 20kDa mass range.

These mass spectra are then automatically compared against a validated database containing the mass spectra of a wide range of bacteria (approximately 7,000 strains as of November 2017). A scoring algorithm determines the closest matches for the sample against the database and assigns a confidence score to the matching organism.

Interferences:
There are a number of plasticisers which have been found to interfere with the performance of MALDI-ToF MS. Due to this plastic tools/consumables are avoided wherever possible.

When plastic use is unavoidable only those products which have been shown to have no effect on the method performance are permitted (e.g. Eppendorf branded pipette tips).

Performance of Method:
The method has undergone validation at ALS Coventry and the data is available for review on site

Result Interpretation:
Results are expressed as the best-matching organism from the database along with a confidence score ranging from 0.000 to 3.000.

A score of 2.000 or higher indicates a confident result to the species level.

A score between 1.700 and 1.999 indicates a confident result to the genus level.

A score below 1.700 indicates that there is no reliable identification available within the database.

In the case of a genus-confident result further work may be required to improve the confidence of the result depending on the organism being tested for. For example, a genus level result (1.7+) is adequate for Salmonella identification but a species level result (2.0+) would be required for Escherichia coli.

In the case of no reliable identification being obtained further interpretation will be required from to determine what further work is required.

Where appropriate the best matching organism will be added as an analyst comment on the analytical report for the sample.

Limitations:
Identification of microbiological organisms by MALDI-ToF MS is reliant upon having a source of living, pure bacteria from which to prepare the target plate. If either of these criteria are not met then further isolation work and incubation time will be required before reliable MALDI-ToF MS results can be obtained.

Confirmations by MALDI-ToF MS are not possible for organisms which are not present within the validated database of known bacteria.

Some bacteria, yeasts or moulds may not produce appropriate mass spectra for identification when prepared with the standard smearing technique. In this case the organism will require an extended extraction technique utilising formic acid to release the target proteins.
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