METHOD STATEMENT

Determinand:
Fungal and Bacterial Identification

Matrix:
Environmental samples (Soils, waters, swabs, contact plates, settle plates, air plates, air strips, etc.)

Principle of method:
The laboratory uses a MALDI-TOF MS (Matrix-assisted laser desorption/ionisation time of flight mass spectrometer) instrument to identify unknown organisms. The instrument analyses the protein structure of an organism and compares it to a reference library to produce an identification. It is referred to as the Biotyper.

Many of the microorganisms identified in the laboratory can also be identified by testing for certain morphological and biochemical properties.

Bacteria can be categorised by carrying out a Gram Stain, and for greater specificity we can make use of commercially available Crystal ID or API kits. Moulds are identified by their growth characteristics on selective agars and by microscopy. Yeasts are categorised as either 'Candida' or 'non Candida' using selective agar.

Sampling and Sample Preparation
Colonies for ID may come from cultures isolated from samples by the laboratory, or from plates submitted to the laboratory.

If the customer has requested screening for a particular organism/genus then an appropriate isolation method should be performed on the sample. For example, for a species of *Klebsiella*, the coliforms method W10 should be used to culture colonies, which should then be identified as per section 7.

If the customer requests screening the sample for unknowns, then several plates should be prepared. The volume analysed will depend on the volume of sample submitted. Generally a 100ml, 10ml and 1ml (further dilutions may be required for dirty samples) portion should be filtered through a membrane and transferred to the surface of an agar plate for each of the following;

Aerobic – YEA/NA/TSA or CBA – 2 days at 30ºC
Anaerobic – CBA – 2 days at 30ºC
Microaerophillic – CBA – 2 days at 30ºC
Yeasts and Moulds – SDA or RBA – 5 days at 25ºC

From all plates, each morphological colony type should be identified.

Interferences:
Fast-growing moulds may mask some smaller colonies or slow growing species. In this case, sub-culturing may be required.
Plasticisers can interfere in the mass spectrometry analysis. Only certified, non-plasticising plastic materials should be used.

Performance of method:
Not applicable for this method.
Analysis may only be carried out by qualified staff, who have received training from a recognised mycology center.

References:

Identification of Pathogenic Fungi. CK Campbell, EM Johnson, CM Philpot, DW Warnock. Health Protection Agency, 1996.