METHOD STATEMENT

Determinand:
Yeasts and Moulds

Matrix:
Raw and Potable waters

Principle of method:
This method relies on the entrapment of yeast and moulds on the surface of a membrane with 0.45µm pores, following the filtration of a known volume of water sample. This membrane is then placed on the selective growth medium Rose Bengal Chloramphenicol and incubated at an appropriate temperature. Chloramphenicol is an antibiotic that suppresses the growth of gram-negative bacteria. Yeast and mould cells take up Rose Bengal dye, which serves to restrict colony size and spreading, thus preventing overgrowth of slow-growing species and assists with the enumeration of small colonies.

Sampling and Sample Preparation:
Once taken, samples should immediately be stored in the dark and kept at a temperature of between 2-8°C for transport to the laboratory. On arrival at the laboratory, samples should be transferred into storage set at these conditions until analysis commences.

Ideally, samples should be analysed on the day of receipt at the laboratory. However, samples can be stored in the above conditions for up to 24 hours prior to analysis.

Interferences:
Chlorine and chloramines are neutralised by adding sodium thiosulphate, which at a concentration of 18mg/l should counteract up to 5mg/l of free and combined residual chlorine.

Rose Bengal photo-oxides to form toxic compounds. Therefore, plates must be stored in the dark and all unnecessary exposure to light must be avoided. The time taken to process the samples and incubate the plates must not exceed two hours.

Spreading mould may overgrow or inhibit slower growing species.

Performance of method:

Range of Application:	0 – 100 cfu/100ml without dilution
Limit of Detection:	1 cfu/100ml
Normal Reporting Level:	0 cfu/100ml

References:
Environment Agency - The Microbiology of Drinking Water (2004).

Oxoid, The Manual, 7th Edition (1995).

Brock et al; Biology of Microorganisms 2000, 9th Edition.