LAB163
R2A Medium

Description
R2A medium was developed to determine the bacterial count in potable waters during treatment and distribution, and has been shown to give significantly higher counts than plate count agar (PCA) or similar high-nutrient media. The standard plate count (SPC) method using PCA provides an enumeration of bacteria which grow best at, or near, body temperature and this estimation at best may correlate to the coliforms present in the sample. However, there will be a population of heterotrophic bacteria which cannot grow at all under the conditions of the SPC method or may grow so slowly that the colonies fail to reach a size detectable to the eye in the 48-h incubation period. In order to enumerate this section of the bacterial population in water, a medium of low nutritional content (R2A) and extended incubation times are required. R2A medium is recommended by the Environment Agency, Methods for the Examination of Waters and Associated Materials, and Standard Methods for the Enumeration of Water and Wastewater.

Formulation

	g/litre
Yeast extract	0.5
Meat peptone	0.5
Casamino acids	0.5
Glucose	0.5
Starch	0.5
Dipotassium hydrogen phosphate	0.3
Magnesium sulphate	0.05
Sodium pyruvate	0.3
Agar	15.0
Grams per litre	**18.0**

Appearance
Powder: fine, free-flowing, homogeneous, buff
Finished medium: clear, opalescent gel

pH: 7.2 ± 0.2

Hazard classification
NR – Not regulated

Method for reconstitution
Weigh 18 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121°C. (If required, bring to the boil to dissolve the agar, and pour into smaller volumes before sterilizing.) Cool to 44-46°C for not more than 3 hours before use. Mix well before dispensing into Petri dishes. Dry the agar surface prior to use.

Inoculation
Pour 15ml into a Petri dish containing 1ml of sample, mix well and allow to set. Pour a further 10ml as an overlay and again allow to set. Alternatively it may be used as a spread plate, inoculating 0.1ml onto the plate and spreading over the entire surface of the medium. It can also be used with membrane filters if required.

Incubation
When plates have set, incubate at 22°C for 5-7 days or 30°C for 3 days. Other incubation temperatures between 20°C and 28°C may be used.

Interpretation
Count all colonies and report the number of bacteria in the original sample as the heterotrophic plate count.

Storage

Dehydrated culture media:	10-25°C away from direct sunlight.
Prepared media:	Plates - 7 days at 2-8°C in the dark
	Capped containers – 3 months at 15-20°C in the dark

Minimum Q.C. organisms
Aeromonas hydrophila NCTC 8049
Pseudomonas fluorescens NCTC 10038

References
Reasoner, D.J., Geldreich, E.E. (1985) A New Medium for the Enumeration and Subculture of Bacteria from potable water. App & Env. Microbiol. Jan. 1985 p. 1-7.

American Public Health Association (1985) Standard Methods for the Enumeration of Water and Wastewater. 16th Edition. American Public Health Association Inc. Washington DC.

Environment Agency: The Microbiology of Drinking Water (2002). Methods for the Examination of Water and Associated Materials.