



04/2023:0169

Sodium pyruvate	0.3 g
Agar	15.0 g
Purified water	to 1000 mL

Adjust the pH so that after sterilisation it is 7.2 ± 0.2 . Sterilise by heating in an autoclave at 121°C for 15 min.

Growth promotion of R2A agar

- **Preparation of test strains.** Use standardised stable suspensions of test strains or prepare them as stated in Table 0169.-1. Seed lot culture maintenance techniques (seed-lot systems) are used so that the viable micro-organisms used for inoculation are not more than 5 passages removed from the original master seed-lot. Grow each of the bacterial strains separately as described in Table 0169.-1. Use buffered sodium chloride-peptone solution pH 7.0 or phosphate buffer solution pH 7.2 to make test suspensions. Use the suspensions within 2 h, or within 24 h if stored at $2-8^\circ\text{C}$. As an alternative to preparing and then diluting a fresh suspension of vegetative cells of *Bacillus subtilis*, a stable spore suspension is prepared and then an appropriate volume of the spore suspension is used for test inoculation. The stable spore suspension may be maintained at $2-8^\circ\text{C}$ for a validated period of time.
- **Growth promotion.** Test each batch of ready-prepared medium and each batch of medium, prepared either from dehydrated medium or from the ingredients described. Inoculate plates of R2A agar separately with a small number (not more than 100 CFU) of the micro-organisms indicated in Table 0169.-1. Incubate under the conditions described in the table. Growth obtained must not differ by a factor greater than 2 from the calculated value for a standardised inoculum. For a freshly prepared inoculum, growth of the micro-organisms must be comparable to that obtained with a previously tested and approved batch of medium.

Table 0169.-1. – Growth promotion of R2A agar

Micro-organism	Preparation of the test strain	Growth promotion
<i>Parasarcosina aeruginosa</i> such as: ATCC 4927 NCIMB 8626 CIP 82.138 NBRC 13275	Casein soyabean digest agar or casein soyabean digest broth $30-35^\circ\text{C}$ 18–24 h	R2A agar < 100 CFU $30-35^\circ\text{C}$ < 3 days
<i>Bacillus subtilis</i> such as: ATCC 6633 NCIMB 8054 CIP 82.62 NBRC 3134	Casein soyabean digest agar or casein soyabean digest broth $30-35^\circ\text{C}$ 18–24 h	R2A agar < 100 CFU $30-35^\circ\text{C}$ < 3 days

Total organic carbon (2.2.44): maximum 0.5 mg/L.

Conductivity. Determine the conductivity off-line or in-line under the following conditions.

EQUIPMENT

Conductivity cell:

- electrodes of a suitable material such as stainless steel;
- cell constant: the cell constant is generally certified by the supplier and is subsequently verified at suitable intervals using a certified reference solution with a conductivity less than $1500 \mu\text{S}\cdot\text{cm}^{-1}$ or by comparison with a cell having a certified cell constant. The cell constant is confirmed if the value found is within 2 per cent of the certified value, otherwise re-calibration must be performed.

Conductometer: accuracy of $0.1 \mu\text{S}\cdot\text{cm}^{-1}$ or better at the lowest range.

System calibration (conductivity cell and conductometer):

- against one or more suitable certified reference solutions;

WATER FOR INJECTIONS

Aqua ad iniectionabile

H₂O

At, 18.02

DEFINITION

Water for the preparation of medicines for parenteral administration when water is used as vehicle (water for injections in bulk) and for dissolving or diluting substances or preparations for parenteral administration (sterilised water for injections).

Water for injections in bulk

PRODUCTION

Water for injections in bulk is obtained from water that complies with the regulations on water intended for human consumption laid down by the competent authority or from purified water. It is produced either:

- by distillation in an apparatus of which the parts in contact with the water are of neutral glass, quartz or a suitable metal and which is fitted with an effective device to prevent the entrainment of droplets; or
- by a purification process that is equivalent to distillation. Reverse osmosis, which may be single-pass or double-pass, coupled with other appropriate techniques such as electro-deionisation, ultrafiltration or nanofiltration, is suitable. Notice is given to the supervisory authority of the manufacturer before implementation.

For all methods of production, correct operation monitoring and maintenance of the system are essential. In order to ensure the appropriate quality of the water, validated procedures, in-process monitoring of the electrical conductivity, and regular monitoring of total organic carbon and microbial contamination are applied.

The first portion of water obtained when the system begins to function is discarded.

Water for injections in bulk is stored and distributed in conditions designed to prevent growth of micro-organisms and to avoid any other contamination.

Microbiological monitoring. During production and subsequent storage, appropriate measures are taken to ensure that the microbial count is adequately controlled and monitored. Appropriate alert and action levels are set so as to detect adverse trends. Under normal conditions, an appropriate action level is a microbial count of 10 CFU per 100 mL when determined by filtration through a membrane with a nominal pore size not greater than $0.45 \mu\text{m}$, using R2A agar, using at least 200 mL of water for injections in bulk and incubating at $30-35^\circ\text{C}$ for not less than 5 days. For aseptic processing, stricter alert levels may need to be applied.

R2A agar

Yeast extract	0.3 g
Protease peptone	0.3 g
Casein hydrolysate	0.3 g
Glucose	0.3 g
Starch	0.5 g
Dipotassium hydrogen phosphate	0.3 g
Magnesium sulfate, anhydrous	0.024 g

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AN Whitehead



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