

## Annex A (normative)

### Composition and preparation of culture media and reagents

#### A.1 Tryptose sulfite cycloserine agar (TSC agar), References[6][11][12]

##### A.1.1 Basal medium

Enzymatic digest of casein	15 g
Enzymatic digest of soya	5 g
Yeast extract	5 g
Sodium disulfite (sodium metabisulfite), anhydrous (Na <sub>2</sub> S <sub>2</sub> O <sub>5</sub> ) (CAS No 7681-57-4)	1 g
Iron(III) ammonium citrate (CAS No.: 1185-57-5)	1 g
Agar	9 g to 18 g
Water	1 000 ml

Suspend the ingredients in the water and dissolve by heating and stirring. Sterilize by autoclaving at (121 ± 3) °C for 15 min. Allow to cool to 45 °C to 50 °C. The basal medium may be stored at (5 ± 3) °C and used within 4 weeks of preparation. To prepare the complete medium the stored medium is remelted by steaming and cooled to 45 °C to 50 °C before adding the cycloserine solution (see [A.1.2](#)).

##### A.1.2 D-cycloserine solution

D-cycloserine (CAS No.: 68-41-7)	4 g
Water to	100 ml

Dissolve the D-cycloserine in the water and filter through a membrane of 0,2 µm pore size. Dispense aseptically into suitable volumes, store at (-20 ± 5) °C and use within 4 weeks of preparation. Alternatively, the dispensed volumes of cycloserine can be stored at (-70 ± 10) °C for a maximum of 12 months.

##### A.1.3 Complete medium

Basal medium ( <a href="#">A.1.1</a> )	1 000 ml
D-cycloserine solution ( <a href="#">A.1.2</a> )	10 ml

Add the D-cycloserine solution to the molten cooled basal medium, mix well and dispense into vented Petri dishes to a depth of at least 5 mm.

The final pH of the medium should correspond to 7,6 ± 0,2 at 25 °C.

Use the prepared plates as fresh as possible on the same day. If storage of the prepared plates is inevitable, store the plates under anaerobic conditions at (5 ± 3) °C and use them within 7 d.