

# Standard Operating Procedure

## Title: Media Preparation in Microbiology Laboratory

### 1.2. **Trypticase Soy Broth (TSB) + 0.5% Tween 80**

Prepare according to manufacturer's directions, adding 0.5% v/v Tween 80. Heating is necessary for dissolving powdered media. Do not overheat or boil.

#### **TSB + 5% v/v Lecithin**

For a solution to contain a 5% v/v Lecithin Supplement, prepare the TSB according to manufacturers' directions. When preparing 1 litre of this media, add 50 mL of Amyl Supplement SP430 to 1L of Distilled Water used for reconstituting the media.

If Amyl Supplement is not available, prepare this medium using 0.5% w/v Lecithin (soaked and blended) and 4% w/v Tween 20 instead of Polysorbate.

### 1.3. **Fluid Thioglycollate Medium (FTM) + 0.5% Tween**

Prepare according to manufacturer's method adding 0.5% v/v Tween 80. It is critical that this solution be brought to the boil with constant stirring before dispensing.

#### **FTM +0 5% v/v Lecithin**

To prepare a solution containing a 5% v/v Lecithin Supplement prepare FTM according to the manufacturer's instructions. When preparing 1 litre of this media, add 50 mL of Amyl Supplement SP430 to 1L of Distilled Water used for reconstituting the media and **ensure that the media is brought to the boil.**

#### **FTM + 10% Tween 80**

To obtain a solution of FTM containing 10% Tween 80 prepare FTM according to the manufacturers directions, add the Tween 80 and stir to mix thoroughly, **bring to the boil** ensuring that both the FTM and Tween are dissolved.

Prior to dispensing into containers ensure that the solutions are thoroughly mixed in order to obtain a uniform content of Tween throughout the solution.

When dispensing into required containers ensure that all bottles are above two-thirds filled. If, when this medium is about to be used, more than the top fifth of the content is Pink, heating on a boiling water bath may restore the medium, until this Pink colour is discharged. This restoration process must only be performed once on each bottle and this must be noted on the bottle and in the log book.

After autoclaving, agitate the bottles whilst cooling to remix the Tween in the medium if necessary, being careful to avoid oxygenating the medium. Unnecessary agitation of this medium is to be avoided.

### 1.4. **EE Broth**

1.4.1. Weigh out required mass of powdered media and add appropriate volume of water into a large plastic beaker. Stir thoroughly to disperse media.

1.4.2. pH sample.

1.4.3. Pour into pre-sterilised 100ml glass bottles and fill with 90ml, (just below shoulder of bottle). Screw caps on loosely.

1.4.4. Place these filled bottles into approximately 5cm of boiling water in a metal beaker on a hotplate. Boil for 30 minutes. Take care when placing bottles into boiling water.