

# Artificial Sputum Medium

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## Method Article

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# Abstract

The artificial sputum medium (ASM) was formulated to mimic the sputum of cystic fibrosis (CF) patients. The intra- and inter-patient variation in the composition of highly viscous CF sputum complicates the achievement of a reproducible culture condition to study microbial biofilms, for example, *Pseudomonas aeruginosa* colonization in the CF lung. However, the ASM is a homogenous non-viscous medium, which can be prepared in bulk quantities using a fixed set of ingredients. The conventional biofilm models always had cells attached to a solid biotic or abiotic surface submerged in a steady-state or continuous flow of culture medium. In contrast, *P. aeruginosa* grows in the CF lung under micro-aerophilic to anaerobic conditions in the form of microcolonies eventually forming a macro-aggregate/colony in which bacteria adhere to each other and to sputum components. Similarly, in ASM, *P. aeruginosa* forms micro- and macro-colonies and showed various phenotypes specific to CF isolates.

## Introduction



## Reagents

Ingredients for 1 litre of ASM: 5 g mucin from pig stomach mucosa (NBS Biologicals), 4 g low molecular-weight salmon sperm DNA (Fluka), 5.9 mg diethylene triamine pentaacetic acid (DTPA) (Sigma), 5 g NaCl (Sigma), 2.2 g KCl (Sigma), 1.81 g Tris base (Sigma), 5 ml egg yolk emulsion (Oxoid), 250 mg each of 20 amino acids (Sigma). Note: 5 g/L casamino acids (Difco) can be used instead of 20 amino acids.

## Equipment

Glasswares, magnetic stirrer, pH meter, autoclave.

## Procedure

1. Take 800 ml of distilled water in a beaker and place it on a magnetic stirring plate.
2. Use a magnetic stirrer to dissolve the ingredients completely.
3. Add mucin, DNA, DTPA, salts NaCl and KCl, and Tris base one after another with constant stirring.
4. Add 250 mg of each amino acid except tryptophan.
5. Stir the mixture continuously to dissolve amino acids.
6. Insert a calibrated pH probe and check the pH of the medium continuously with constant stirring.
7. Adjust the pH using Tris base until it stabilizes at 7.0.
8. Make up the volume of the medium to 1000 ml.
9. Sterilize the medium in an autoclave at 110°C for 15 minutes.
10. Cool the sterilized medium and add filter-sterilized tryptophan (250 mg/L of medium) from the stock.
11. Add 5 ml of egg yolk emulsion.
12. Maintain sterile conditions for steps 10 and 11.
13. Mix the medium well and store in a refrigerator for several months. Note: For preliminary/routine experiments, casamino acids could also be used at 5 g/L of medium, instead of individual amino acids.

# Troubleshooting

Stabilization of pH is an important factor in the preparation of ASM due to the presence of amino acids.

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## Figures

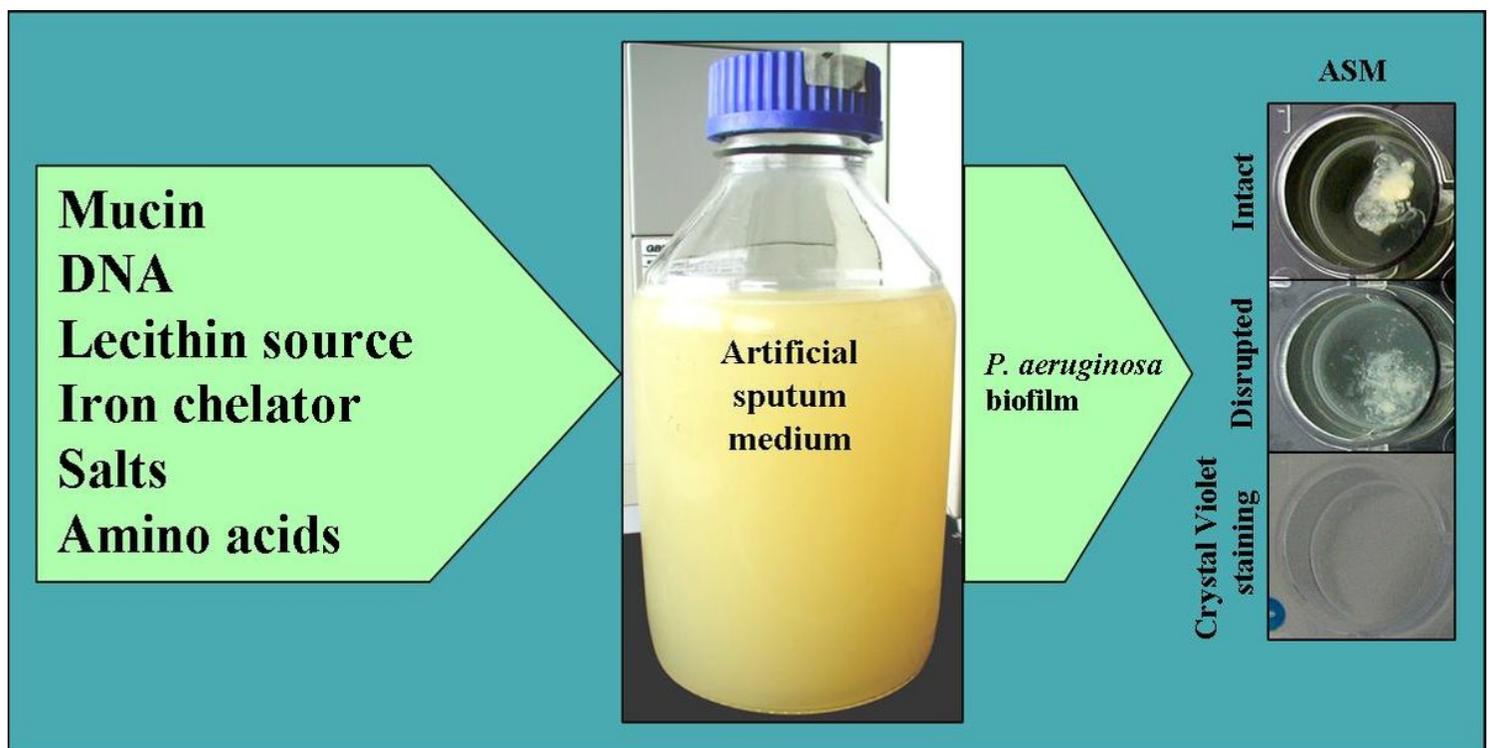


Figure 1

Introduction Figure Artificial sputum medium Intact: intact biofilm in ASM, Disrupted: ASM-grown biofilm disrupted by repeated pipetting. No colouration in the crystal violet staining of the culture well shows that

ASM-grown biofilm do not attach to the surface.