ABSTRACT
Creating an artificial saliva solution for oral biofilm growth, specifically dental calculus.

This is a modified version of Sissons et al. 1991.

GUIDELINES
This protocol explains how to mix 1000 mL of artificial saliva.
MATERIALS

Chemicals
- Mucin from porcine stomach (Type III)
- Trypticase peptone
- Proteose peptone
- Yeast Extract
- KCl
- NaCl
- CaCl$_2$
- Na$_2$HPO$_4$
- NaHCO$_3$
- Hemin
- Menadione
- Urea
- L-Arginine

Equipment
- 1000 mL beaker
- 100 mL graduated cylinder
- 2 x 1000 mL bottle
- Magnetic stirrer (with heating element)
- Autoclave
- pH measure
PROTOCOL MATERIALS

- **Mucin from porcine stomach (Type III)** Merck MilliporeSigma (Sigma-Aldrich) Catalog #M1778

**Step 2**
- **CaCl2** Contributed by users
- **NaOH** Contributed by users

**Step 3**
- **Oxoid® Proteose Peptone** Thermo Fisher Catalog #LP0085B
- **NaCl** Contributed by users
- **NaHCO3** Contributed by users

**Step 5**
- **Oxoid® Proteose Peptone** Thermo Fisher Catalog #LP0085B

**Step 7**
- **Urea** Contributed by users
- **L-Arginine** Contributed by users Catalog #A5006
- **Bacto Yeast Extract** Becton Dickinson (BD) Catalog #10071-028
- **Menadione** Contributed by users

BEFORE START INSTRUCTIONS

Mix the solution under a fumehood. It can smell pretty bad.
Also have the incubator under a fumehood, if possible. I had no such luxury...

1. Add 300 mL distilled (or deionized) dH₂O to a 1000 mL beaker, with stirring and heat 60 °C.

2. Add:

   - 2.5 g **Mucin from porcine stomach (Type III)** Becton Dickinson (BD) Catalog #M1778
   - 5 g **Trypticase® Peptone** Becton Dickinson (BD) Catalog #211921
Let the reagents completely dissolve before continuing to the next step.
4. Add the remaining 700 mL distilled (or deionized) H₂O.

5. Adjust to pH 7 with NaOH Becton Dickinson (BD) and stirring.

6. Transfer to two 1000 ml bottles, so around half of each bottle is filled.
Autoclave at 121 °C, 1 Bar for 00:15:00 minutes

Safety information

Do NOT screw bottle caps on tightly before autoclaving.

Loosely screw the caps on the bottles or cover the tops with foil.

Once the solution has cooled, combine solutions to a single 1000ml bottle, and add:

- 1 mg Menadione Becton Dickinson (BD)
- 0.3 g Urea Becton Dickinson (BD)
- 0.17 g L-Arginine Becton Dickinson (BD) Catalog #A5006

Safety information

These should be filter-sterilised as there are no more decontamination steps for the artificial saliva.

Use clean gloves and take precautions not to contaminate the artificial saliva with bacteria or starch.

Store in fridge at ca. 4 °C

Occasionally test the pH of the solution to ensure it stays around 7